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New data on *Gaidropsarus granti* (Regan, 1903) (Gadiformes: Lotidae) from the Mediterranean Sea, with emphasis on its parasites

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SUMMARY: One adult male Azores rockling *Gaidropsarus granti* was captured by trammel nets at a depth of about 250 m near the coast of Arbatax (Sardinia, Italy) in early March 2007. This new report confirms a wide bathymetric range for this species. Macroscopic and microscopic analysis of the gonad showed a spent testis at a postspawning stage, with a weak residual spermatogenetic activity. Several body parts of Natantia (Crustacea: Decapoda) were detected in its stomach contents. Different developmental stages of 91 parasite specimens belonging to Arthropoda (Gnathiidae) and Nematoda (Anisakidae, Cystidicolidae and Philometridae) were found in its mouth and gills, and body cavity, respectively. Myxozoan spores were found in the gallbladder. Male and female nematodes of the genus *Ichthyofilaria* are reported for the first time from the Mediterranean Sea, and a very rare male of this genus is reported for the second time in the world. Parasitological results indicated that this Atlantic migrant probably entered the Mediterranean as an adult, suggesting for a non-indigenous species the possibilities of entering with natural parasites and/or acquiring native parasites in the introduced range.

Keywords: *Gaidropsarus granti*, Lotidae, Atlantic migrant, Mediterranean Sea, parasites, non-indigenous species.

RESUMEN: NUEVOS DATOS ACERCA DE *GAIDROPSARUS GRANTI* (REGAN, 1903) (GADIFORMES: LOTIDAE) EN EL MEDITERRÁNEO OCCIDENTAL, CON ÉNFASIS SOBRE SUS PARÁSITOS. – Un ejemplar macho adulto de barbada de las Azores fue capturado con trasmallo alrededor de 250 m de profundidad cerca de la costa de Arbatax (Cerdeña, Italia) a principios de marzo de 2007. Esta nueva cita confirma que *G. granti* presenta un amplio rango de distribución batimétrica. El examen macroscópico y microscópico de las gónadas mostró un testículo en estadio de postpuesta con una actividad espermatogénica reducida. En su contenido estomacal se encontraron varios restos de Natantia (Crustacea: Decapoda). Diversos estadios de desarrollo de 91 especímenes de parásitos pertenecientes a Arthropoda (Gnathiidae) y Nematoda (Anisakidae, Cystidicolidae y Philometridae) fueron encontrados en su boca y branquias, y cavidad corporal, respectivamente. Esporas de myxozoos fueron además encontradas en la vesícula biliar. Se encontraron por primera vez en el Mediterráneo ejemplares macho y hembra del nematodo *Ichthyofilaria* sp., y además el rarísimo macho de este género se cita por segunda vez en el mundo. Los resultados parasitológicos mostraron que este migrador atlántico probablemente entró en el Mediterráneo como adulto, sugiriendo que especies no indígenas pueden entrar con sus parásitos naturales y/o adquirir parásitos nativos en nuevos hábitats.

Palabras clave: *Gaidropsarus granti*, Lotidae, migrador atlántico, mar Mediterráneo, parásitos, especies no indígenas.

INTRODUCTION

In the Mediterranean Sea, the rate of reports of non-indigenous species (NIS) has considerably increased in recent decades, involving the possibility of significant ecological and economic impacts (Galil, 2000). With regard to fish, Quignard and Tomasini (2000) estimated that at least 26 Atlantic species entered the Mediterranean fairly recently, and this number is likely to increase dramatically year by year, as new records of NIS migrating from both the boreal and tropical part of the Atlantic Ocean are continuously reported throughout the Mediterranean basin. The majority of these immigrants are Osteychthyes, but less than a half of these species can be considered as established (*sensu* Golani *et al.*, 2002).

According to Froese and Pauly (2007), three species of the genus *Gaidropsarus* Rafinesque, 1810 (Gadiformes: Lotidae) commonly occur in the Mediterranean Sea: *Gaidropsarus biscayensis* (Collett, 1890), *G. mediterraneus* (Linnaeus, 1758), and *G. vulgaris* (Cloquet, 1824). The Azores rockling, *Gaidropsarus granti* (Regan, 1903), is mainly known from the Azores (from which the common name is derived) and the Canary Islands (Svetovidov, 1973; Dooley *et al.*, 1985; Cohen, 1990). More recently, its presence was reported by Bañón *et al.* (2002) from Galician waters (eastern Atlantic, Spain). Although generally considered as rare, this fish still has a controversial bathymetric distribution range. In fact, while Svetovidov (1986) stated that it probably lives on rough grounds at depths ranging from 20 to 50 m, Brito *et al.* (2002) reported this species as demersal from deep banks and upper bathyal [as cited by Svetovidov (1973) based on reference from Great Canaries Island by Steindachner (1891) as *Onus guttatus*]. Food habits of *G. granti* are also poorly known, as shrimps and prawns are so far the only food items recorded in its diet (Froese and Pauly, 2007).

The first record of this species in the Mediterranean Sea was documented by Zachariou-Mamalinga (1999) from the southeastern Aegean Sea (Rhodes, Greece) where in 1995 a single specimen was captured on a sandy bottom at a depth of about 360-400 m. Afterwards, as reported by Golani *et al.* (2002), the occurrence of *G. granti* was confirmed for both the Ligurian (Orsi-Relini, pers. comm.) and Adriatic Seas (Bello, pers. comm.). In the light of these two records, these authors argued that it was not possible to determine whether this species is indigenous or exotic to the Mediterranean. Subsequently, Mura and Cau (2003) found another specimen of the Azores rockling on a trawling bottom at a depth of about 120-136 m in the Sardinian Channel (central western Mediterranean, Italy).

However, to date, there is a general lack of ecological data on this species in the Mediterranean Sea, as well as on its parasites. Therefore, the capture of a new specimen of *G. granti* off the eastern coast of Sardinia represented a rare opportunity to collect additional information on its range extension, biology and gonadal activity. Moreover, its parasites and their potential significance as biological tags were investigated to determine whether the fish was indigenous or not to the Mediterranean.

MATERIALS AND METHODS

On 5 March 2007, one adult specimen of *Gaidropsarus granti* was captured by trammel nets off Capo di Monte Santu, a promontory along the northern shore near Arbatax (Sardinia, central western Mediterranean, 40°03.9’N 9°46.7’E) at a depth of about 250 m (Fig. 1). The fish was photographed (Fig. 2), then immediately frozen at -20°C. It was identified following Svetovidov (1986) and its morphometric and meristic characters were recorded according to Strauss and Bond (1990). The number of vertebrae was counted using radiography, then the fish was carefully dissected. Its stomach contents were weighed and then stored in 70% ethanol. After sex determination (male), the maturity stage of the testis was assessed macroscopically according to Holden and Raitt (1974), then the gonad was fixed in 5% buffered formalin for subsequent histological analysis. Afterwards, the testis was dehydrated in

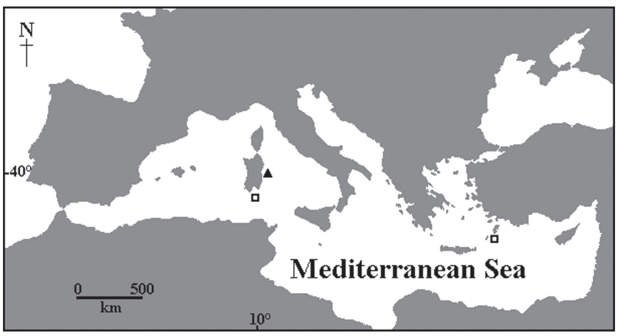

FIG. 1. – Distributional map of *Gaidropsarus granti* documented records in the Mediterranean Sea. Black triangle indicates the site of collection of the present specimen. Empty squares indicate localities of previously recorded specimens.

FIG. 2. – *Gaidropsarus granti* (Regan, 1903). Department of Animal Science, University of Sassari (Italy) ichthyological collection (ref. number DSZ-03/07).

an ascending ethanol series, embedded in resin, and semi-thin sections of 3 μm were stained with sodium iodoeosine and toluidine blue following Dominici's method modified for methacrylate (Mazzi, 1977). The identification of developmental stages of testicular germinal cells was based on the spermatogenic differentiation scale proposed by Grier (1981).

Body surface, mouth, gills, body cavity and all internal organs of the fish were examined for metazoan parasites and fresh scrapings of the organs were observed microscopically for protozoan parasites. The parasites found were processed for identification according to standard protocols (Berland, 1984; Roberts, 1989). For final taxonomic classification, drawings of the parasites were made using a camera lucida and photomicrographs were taken with a digital camera (Olympus CZ2000) connected to a microscope. Measurements were made using the Olympus DP-Soft 5.0 software. The parasites were identified at the lowest possible taxonomic level, counted and stored in 70% ethanol. Type I and Type II third stage larvae of *Anisakis* sp. (*sensu* Berland, 1961) were identified at species level using RFLP genetic analysis and the molecular keys devised by D'Amelio *et al.* (2000), implemented by Pontes *et al.* (2005).

The fish was deposited in the ichthyological collection of the Department of Animal Science at the University of Sassari (registration code DSZ-03/07).

RESULTS

Description of the specimen examined

The fish was 319 mm in total length, and weighed 186.7 g. Its morphometric and meristic characters, compared with those of the two other specimens previously documented in the Mediterranean Sea, are presented in Table 1. The measurements and the colour pattern of the new reported specimen agree with Svetovidov (1986), Zachariou-Mamalinga (1999), and Mura and Cau (2003), except for the number of vertebrae, which differs from the observations of the former author.

Gonadal status

Macroscopic analysis of the testis showed that it was at a spent, postspawning stage (stage 8 of Holden and Raitt, 1974). Macroscopically it appeared flaccid and reddish in colour. Subsequent histological analyses also revealed a collapsed testicular tissue with most of the seminiferous tubules empty and a thickening of the interstitial matrix (stage VI of

TABLE 1. – Morphometric and meristic data of *Gaidropsarus granti* specimens recorded in the Mediterranean Sea.

	Present specimen	Zachariou-Mamalinga (1999)	Mura and Cau (2003)
Morphometric characters (mm)			
Total length	319	310	245
Standard length	287	280	220
Head length	60	66.5	56
Snout length	17.5	15	-
Predorsal length	65	61.1	46.5
Preanal length	150	145	-
Eye diameter	11.2	9.1	9.1
Interorbital width	9	11.3	9
1st dorsal fin length	8.5	9.6	7.1
Caudal peduncle length	7.7	7.4	5.7
Caudal peduncle minimum height	19.7	19	15
Meristic characters			
2nd dorsal fin rays	60	56	55
Anal fin rays	49	47	47
Pectoral fin rays	20	20	20
Ventral fin rays	7	7	7
Vertebrae	47	-	47
Pyloric caeca	6	-	-

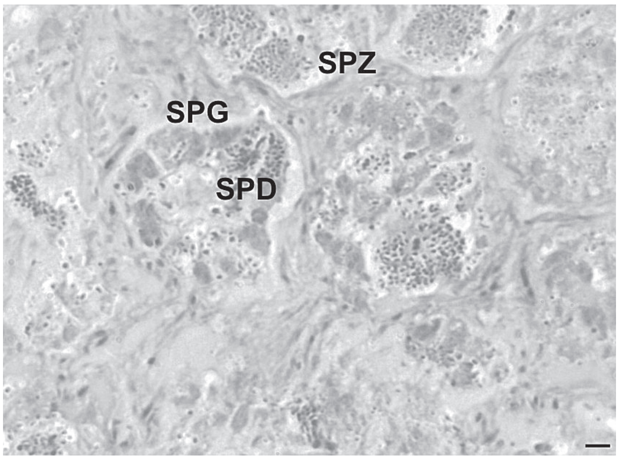

FIG. 3. – *Gaidropsaurus granti* testis section showing a weak, residual spermatogenesis activity. Spermatogonia (SPG), spermatids (SPD) and few spermatozoa (SPZ) are visible. Bar=50 µm.

Grier, 1981). Microscopic observations evidenced a weak residual spermatogenetic activity of the gonad. Immature germinal cells like spermatogonia, together with few spermatides and a small number of spermatozoa were still visible (Fig. 3).

Stomach contents and parasites

The stomach contents comprised several body parts of Natantia (Crustacea: Decapoda), but their advanced state of digestion did not allow a better taxonomic identification.

Different developmental stages of 91 specimens of metazoan parasites belonging to Arthropoda (Gnathiidae) and Nematoda (Anisakidae, Cystidicolidae and Philometridae) were detected in its mouth, gills and body cavity. Several myxozoan spores were found in the gallbladder. The parasite list, with indication of location, stage of development, and intensity of infection (*sensu* Margolis *et al.*, 1982) is presented in Table 2. The intensity of infection of myxozoans was semiquantitatively evaluated following a scale from + to ++++++, based on the number of parasites per microscopic field at 200×, as described by Alvarez-Pellitero *et al.* (1995).

DISCUSSION

This is the third documented record of *G. granti* from the Mediterranean Sea and the second from Italian waters, adding new data on the presence of this species in the central western part of the basin. Svetovitov (1986) reported that *G. granti* lives at depths ranging between 20 and 50 m around the Azores and the Canary Islands, while Brito *et al.* (2002) reported this species as demersal from deep banks and upper bathyal. It is worth mentioning that either the present specimen and the others previously documented from the Mediterranean were captured at deeper depths. This allows us to hypothesise that this fish occurs over a wide bathymetric range. The higher number of vertebrae [47 vs. 44 reported by Svetovitov (1986)] of the specimen described is consistent with the observations reported by Mura and Cau (2003). However, a constant variability in this meristic character is common to the sub-family Lotinae (Svetovitov, 1986).

Up to now, no data on the reproductive period of this species are reported in the literature. The present specimen, caught in late winter, appears to be a male at a postspawning stage. The presence of rests of Natantia in the stomach of the specimen examined confirms the observations on the feeding habits of this fish reported by Svetovidov (1986).

As far as parasites found are concerned, praniza larvae of Gnathiidae cannot be identified since the taxonomy of this group is based entirely on free-living adult males (Cohen and Poore, 1994). Gnathiidae have been reported in most parts of the world oceans. These parasites are particularly abundant in coral reefs, being the most common external parasites on coral reef fish (Grutter and Poulin, 1998)

TABLE 2. – List of the parasites found in the *Gaidropsaurus granti* specimen examined.

Location	Phylum	Class	Order	Family	Genus	Species	Stage	Intensity
Mouth/gills	Arthropoda	Malacostraca	Isopoda	Gnathiidae	n.d.	n.d.	Praniza	25
Body cavity	Nematoda	Secernentea	Ascaridida	Anisakidae	*Anisakis*	*A. physeteris*	3rd stage larva	1
Body cavity	Nematoda	Secernentea	Ascaridida	Anisakidae	*Anisakis*	*A. simplex* s.str.	3rd stage larva	1
Body cavity	Nematoda	Secernentea	Ascaridida	Anisakidae	*Hysterothylacium*	*H. aduncum*	4th stage larva	6
Body cavity	Nematoda	Secernentea	Spirurida	Cystidicolidae	n.d.	n.d.	3rd stage larva	2
Body cavity	Nematoda	Secernentea	Spirurida	Philometridae	*Ichthyofilaria*	n.d.	Gravid female	51
							Adult male	5
Gallbladder	Myxozoa	Myxosporea	Bivalvulida	Ceratomyxidae	*Leptotheca*	n.d.	Spores	++++++(1)

n.d., not determined; (1) see Alvarez-Pellitero *et al.* (1995) for details.

and the main food item for many cleaner fish (Grutter, 2002).

Anisakis physeteris Baylis, 1923 is a common parasite in gadiforms from the central and western Mediterranean Sea and the Atlantic Ocean (Mattiucci *et al.*, 2004; Farjallah *et al.*, 2006; Valero *et al.*, 2006a, b). The high prevalence of this species from the sampling area is probably related to the trophic ecology of the fish hosts and the migration routes of the sperm whale *Physeter catodon* L., the definitive host of this parasite (Mattiucci *et al.*, 2004).

Anisakis simplex sensu stricto Nascetti, Paggi, Orecchia, Smith, Mattiucci and Bullini, 1986 is known to have a worldwide distribution mainly with a benthic or demersal life cycle (Mattiucci and Nascetti, 2006). In the Mediterranean, this species has been reported only in epi-mesopelagic fish, such as *Micromesistius poutassou* (Risso, 1827), *Scomber japonicus* Houttuyn, 1782, *Scomber scombrus* L., 1758 and *Trachurus trachurus* (L., 1758) from the Alboran Sea (Abollo *et al.*, 2003; Farjallah *et al.*, 2007), in *Thunnus thynnus* (L.) from the South Tyrrhenian (Mattiucci *et al.*, 1996), and in *S. scombrus* from the North African coast (Farjallah *et al.*, 2008). Recently, Farjallah *et al.* (2007) reported *A. simplex* s.str. also in *Merluccius merluccius* (L., 1758) from Gibraltar (between Tanger and Tétouan). The presence of *A. simplex* s.str. in some individual hosts from the western Mediterranean seems to be the result of a migration of these fish from the Atlantic into the western area of the Mediterranean (Mattiucci and Nascetti, 2006; Mattiucci *et al.*, 2008), mixing Atlantic and Mediterranean populations. This fact was also confirmed by homogenous genetic structure and high levels of gene flow between western Mediterranean and Atlantic populations of *T. trachurus*, with only a slight differentiation from eastern Mediterranean populations [Ionian and Aegean Seas (Cimmaruta *et al.*, 2008)]. The present report is the first of *A. simplex* s.str. parasitising a demersal fish from the Mediterranean proper (i.e. not close to Gibraltar). Larvae of this parasite are reported to survive for several years (Smith, 1984) and are considered reliable biological tags (e.g. Mattiucci and Nascetti, 2006; Mattiucci *et al.*, 2004, 2008). In fact, larval stages have to await ingestion by a definitive host to attain maturity and reproduce, which makes a long life-span important. The occurrence of *A. simplex* s.str. in the examined specimen of *G. granti* probably indicates that this individual was not native to the Mediterranean, but entered from the eastern central Atlantic through Gibraltar, retaining some of its natural parasites. Considering that third stage larvae of anisakids are transmitted among paratenic hosts through the food web (Anderson, 2000), the possibility that the specimen examined was infected in the Mediterranean by preying on Atlantic migrating fish is extremely remote. In fact, food habits of *G. granti* lead us to exclude for this species the chance of preying on some adult Atlantic migrant fish host infected by *A. simplex* s.str. (i.e. *M. merluccius*, *M. poutassou*, *S. scombrus*, *T. thynnus* or *T. trachurus*). This hypothesis is also confirmed by the total absence of *A. simplex* s. str. larvae in a demersal fish like *M. merluccius* from the proper Mediterranean (Mattiucci *et al.*, 2004).

The unidentified cysticolid larvae may be related to *Cristitectus congeri* Petter, 1970, whose adult stages were found in *Conger conger* (L., 1758) from the northeastern Atlantic (Petter, 1970; Quinteiro *et al.*, 1989; Saraiva *et al.*, 2000). Similar larvae have been found in various fish species along the northeastern Atlantic coast (Petter, 1970; Sanmartín *et al.*, 2000a,b; Alvarez *et al.*, 2002) and the western Mediterranean (Culurgioni *et al.*, 2006).

Adult males and females of the philometrid nematode *Ichthyofilaria* sp. are reported for the first time from the Mediterranean. This genus comprises five species (Køie, 1993; Timi *et al.*, 2001): *I. argentinensis* Incorvaia, 1999 found in the swimbladder of *Merluccius hubbsi* Marini, 1933 (Merlucciidae) from Argentina; *I. canadensis* Appy, Anderson and Khan, 1985, found in the mesenteries and surface of the liver of the Zoarcidae *Lycodes lavalaei* Vladykov and Tremblay, 1936 and *L. vahlii* Reinhardt, 1831 from Atlantic Canadian waters; *I. dasycotti* Yamaguti, 1935, in *Dasycottus setiger* Bean, 1890 (Cottidae) and *I. japonica* Moravec and Nagasawa, 1985 found in the abdominal cavity of *Sebastes schlegelii* Hilgendorf, 1880 (Sebastidae), both from Japanese waters; and finally Køie (1993), included in the genus *Ichthyofilaria* also *Philometra bergensis* Wülker, 1930, a parasite of *Molva dypterygia* (Pennant, 1784) (Lotidae) from the northeastern Atlantic. Adult males of this genus were previously reported only by Køie (1993). Both males and females of the specimens found were very similar to *I. bergensis*, although at the moment some differences in a number of morphometric characters do not allow them to be ascribed to this species. At present, the recent finding of more *I. bergensis*-like nematodes in the body cavity of other gadiforms from the

Mediterranean (Merella, pers. observ.) do not allow us to use this parasite as a biological tag to establish the non-indigenous origin of the *G. granti* specimen examined.

The spores of *Leptotheca* sp. were similar to those of *Leptotheca elongata* Thélohan, 1895 (=*Leptotheca polymorpha* Labbé, 1899), species previously reported in the gallbladder of various gadiform fish hosts from the Atlantic and the Mediterranean (Kudo, 1920; Lubat *et al.*, 1989). This species is characterised by a high morphometric variability and a definite identification will be possible only after further biomolecular and morphometric studies on the collected material.

To our knowledge, in literature there is no information about the parasites of *G. granti*, so the present paper is the first account of the parasites of this species. On the other hand, there is a consistent number of papers reporting parasites from congeners (e.g. Klimpel *et al.*, 2001 and references therein). Notwithstanding, most of the parasites herein reported are new for *Gaidropsarus* spp., apart from *A. simplex* (identified solely as Type I larva) reported from *G. argentatus* (Reinhardt, 1837) off the Faroese coast (Klimpel *et al.*, 2001), and *L. elongata*, found in the gallbladder of *Motella tricirrata* (=*Gaidropsarus vulgaris*) from the Atlantic and Mediterranean French coasts (Kudo, 1920).

Colautti *et al.* (2004) gave a broad review of the invasion literature, suggesting caution in the uncritical acceptance of the enemy release hypothesis (ERH). This hypothesis points that the abundance or impact of some NIS is related to the scarcity of natural enemies in the introduced range compared with the native one. In general, all the parasites found in the examined host have been reported from both Atlantic and Mediterranean fish hosts or from exclusively Atlantic hosts (i.e. *A. simplex* s.str.). Therefore, it is very difficult to establish whether the parasite assemblage found was mainly composed of natural or native parasites acquired in the new habitat. However, the present results show that the ERH cannot be uncritically accepted, in accordance with Poulin and Mouillot (2003), and Colautti *et al.* (2004).

To sum up, the present report provided some interesting insights into the presence of *G. granti* in the Mediterranean Sea, where its biogeographical range expansion can be favoured by pelagic eggs (common to all *Gaidropsarus* species) or juveniles passing through the Strait of Gibraltar (Zachariou-Mamalinga, 1999; Mura and Cau, 2003). Nevertheless, in view of the parasites found, it is likely that the fish examined entered the Mediterranean at an adult stage. Whatever its migratory stage was, however, from a zoogeographical point of view Sardinia confirms its key role as a crossroads of exotic fish species in the central western part of this basin. Actually, in recent years, the occurrence of several other Atlantic (Andaloro *et al.*, 2005; Pais *et al.*, 2005; Follesa *et al.*, 2006), as well as Lessepsian migrants (Pais *et al.*, 2007), has been documented from the same geographical area.

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