

HELMINTHOLOGIA, 53, 4: 385 - 390, 2016

Research Note

**Report of *Enodiotrema megachondrus* (Looss, 1899) Looss, 1901
(Digenea: Plagiorchiidae) in a green turtle *Chelonia mydas* Linnaeus, 1758
(Testudines, Cheloniidae) from Brazil**M. R. WERNECK^{1,3,*}, L. MODOLO CONTI², B. BERGER²

¹BW Veterinary Consulting. Rua Ponciano Eugênio Duarte 203, Centro, Zip Code: 11680-000, Ubatuba, São Paulo State, Brazil,
*E-mail: max@bwvet.com.br; ²CTA – Environmental services. Address: Rua Saturnino Rangel Mauro 283, Pontal de Camburi, Vitória,
Zip Code: 29062-030, Espírito Santo State, Brazil

Article info

Received December 17, 2015
Accepted February 25, 2016

Summary

This paper describes the occurrence of *Enodiotrema megachondrus* (Looss, 1899) Looss, 1901 in a juvenile green sea turtle (*Chelonia mydas* Linnaeus, 1758) found on the coast of Brazil. This parasite has been described in *Caretta caretta* from Egypt, France, the Mediterranean Sea, the Madeira Archipelago, the Adriatic Sea and the USA, in *C. mydas* from Egypt and the USA, in *Eretmochelys imbricata* from Cuba, in *Lepidochelys olivacea* from Mexico and Costa Rica and in *Lepidochelys kempii* from USA. This note represents the first report of *E. megachondrus* in a green sea turtle in the South-West Atlantic Ocean.

Keywords: Brazil; *Enodiotrema megachondrus*; green turtle; parasites; Plagiorchiidae; Trematodes

Introduction

The family Plagiorchiidae groups a large number of digenetic parasites found in amphibians, reptiles, birds and mammals in different parts of the world. Since its creation, this family has undergone systematic changes, reflecting an enormous taxonomic challenge in grouping the approximately 150 genera (Tkach, 2008).

Looss (1899) erected the genus *Enodia* = *Enodiotrema* (Type species *Enodia megachondrus* Looss 1899) and according to Blair & Limpus (1982), this genus has more than six species: *E. reductum* Looss, 1901; *E. instar* Looss, 1901; *E. acariaeum* Looss, 1902; *E. microvitellatus* Chattopadhyaya, 1970; *E. schikhobalovae* Gupta & Mehrotra 1976 and *E. carettae* Blair & Limpus 1982.

Although these parasites are carried out at various locations in the world, on different hosts (Santoro *et al.*, 2010), None of these species have previously been reported in hosts found in the South-West Atlantic Ocean (Fernandes & Kohn, 2014). The aim of this note is report the first occurrence of *E. megachondrus* in a juvenile *Chelonia mydas* Linnaeus, 1758 from Brazil.

Materials and Methods

In September 2014, a *C. mydas* female (34.2 cm curved carapace length [LCC], weight 3.5 kg) was found on Barrinha beach – São Francisco de Itabapoana city (21°23'27.685"S and 40°58'57.928"W) the State of Rio de Janeiro, Brazil. The animal was found died in the beach and the necropsy was performed immediately. Eighteen *E. megachondrus* was found on necropsy, in small intestine, it was fixed in 70 % alcohol, stained with carmine and cleared with eugenol. Morphometric (in millimeters) data were determined with the aid of an image analysis program (ImageJ, National Institutes of Health). Drawing was made using a drawing tube. The helminth collected was deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC number 36738) Rio de Janeiro State, Brazil. Analyses of the parasites were authorized by federal licenses for activities with scientific purposes (SISBIO 30600-1 and 9329-1).

The identification keys of genera by Tkach (2008), the original descriptions by Looss (1899) and papers by Gupta & Mehrotra

(1976) and Looss (1902) were used for the characterization of the specimen. Morphological and morphometric data from, Looss (1899), Groschaft *et al.* (1977) and Santoro & Morales (2007) were used for the purposes of comparison.

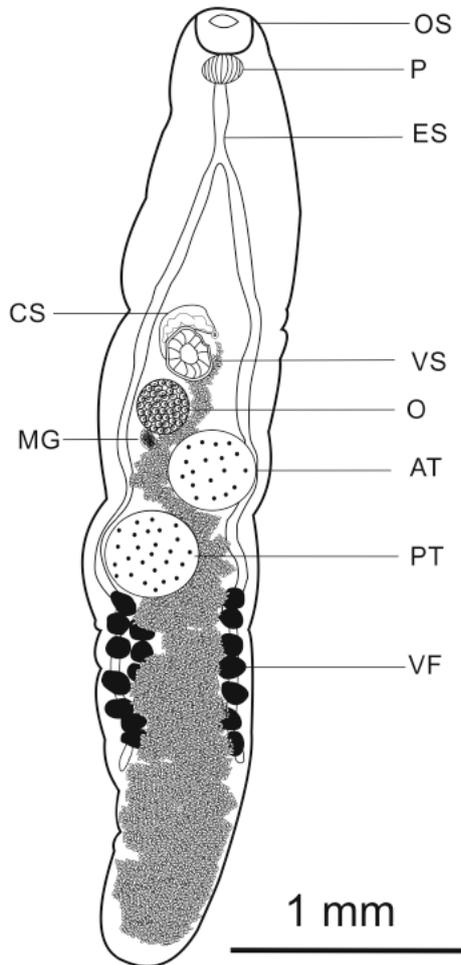


Fig. 1. *Enodiotrema megachondrus* (Looss, 1899) Looss, 1901 (Digenea: Plagiiorchiidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines, Cheloniidae) from Brazil, ventral view. Legend: OS: Oral sucker; P: Pharynx; ES: Esophagus; CS: Cirrus sac; VS: Ventral sucker; O: Ovary; MG: Mehlis' gland; AT: Anterior testis; PT: Posterior testis; VF: Vitelline follicles

Description (Fig. 1, Fig. 2 and Table 1): Body thin with slightly rounded extremities and a slight constriction at the level of the testicles, anterior portion of body covered with small spicules that are absent in the posterior portion; oral sucker terminal (Fig. 2A, B); esophagus slightly sinuous (Fig. 2A); pharynx present; cecal bifurcation closer to anterior region of body (Fig. 2A), caeca follow a slightly sinuous trajectory to the area of the vitelline follicles, at which points continue a short distance and terminate near the end of the vitelline fields; acetabulum anterior to the ovary and quite distanced from the cecal bifurcation; ovary rounded, occupying region between acetabulum and testicular area (Fig. 2C); Mehli's

gland posterior to ovary; both testicles rounded and occupying a diagonal position (Figs. 1 and 2C); cirrus sac present and anterior to acetabulum, occupying a transverse position to the body; uterus replete with eggs and occupying nearly the entire posterior region of the body, its ducts follow a trajectory toward the anterior region of the body, near the testicular region (Figs. 1 and 2E, F), the ducts narrow, passing between the testicles, ovary, Mehli's gland and, near the acetabulum, project to the genital pore near the aperture of the cirrus sac (Fig. 2C – E); vitelline follicles ventral to caeca and rounded, after the testicles region, right follicles ($n = 8$ to 13) and left follicles ($n = 8$ to 11); eggs with elliptical shape and no filament (Fig. 2F).

Previous records: In *C. caretta* from Egypt (Braun, 1901; Looss, 1899); France (Euzet & Combes, 1962); Italy (Manfredi *et al.*, 1998); Mediterranean (Aznar *et al.*, 1998; Santoro *et al.*, 2010); Madera (Valente *et al.*, 2009), Adriatic sea (Gracan *et al.*, 2012) and USA (Greiner, 2013); *C. mydas* from Egypt (Looss, 1902), USA (Greiner, 2013) and Brazil (Present report); *E. imbricata* from Cuba (Groschaft *et al.*, 1977); *L. olivacea* from Mexico (Pérez- Ponce de León *et al.*, 1996; Vivaldo *et al.*, 2006) and Costa Rica (Santoro & Morales, 2007) and *L. kempii* from and USA (Greiner, 2013).

Remarks

Looss (1899) describes the genus *Enodia* (species type *E. megachondrus* Looss, 1899, p. 709-710) based in only one specimen collected in the intestine of *Testudo graeca*. This fact caught the attention of Looss in 1902, that casts doubt regarding the identity of the hosts of the original description (see Looss, 1902 p. 509) and the same author confirms the finding in juveniles of *C. mydas* and *Thalassochelys Corticata* (*Caretta caretta* Linnaeus, 1758) from the Adriatic Sea (Looss, 1902). The author subsequently described *E. reductum* and *E. instar* (Looss, 1901) and, in the following year, offered a better description of the two species as well as describing *E. acariaeum* Looss, 1902 (Looss, 1902).

Enodiotrema megachondrus is a generalist parasite described in *C. mydas* (Looss, 1902; Greiner, 2013; presente report), *C. caretta* (Braun, 1901; Looss, 1901, 1902; Sey, 1977; Aznar *et al.*, 1998; Manfredi *et al.*, 1998; Valente *et al.*, 2009; Santoro *et al.*, 2010; Gracan *et al.*, 2012; Greiner, 2013), *Eretmochelys imbricata* Linnaeus, 1766 (Groschaft *et al.*, 1977), *Lepidochelys olivacea* (Eschscholtz, 1829) (Vivaldo *et al.*, 2006; Santoro & Morales, 2007) and *Lepidochelys kempii* (Garman, 1880) (Greiner, 2013) in different regions of the world. Although *E. megachondrus* has been described in different hosts, this species has been studied better in *C. caretta* hosts, especially in regions of Europe (Aznar *et al.*, 1998; Manfredi *et al.*, 1998; Valente *et al.*, 2009; Santoro *et al.*, 2010; Gracan *et al.*, 1012).

The analysis of 54 individuals of *C. caretta* (CCL range: 34 to 69 cm) from Spain revealed a prevalence of 96 % (54/56) and mean intensity of 74.5 (range: 1 to 680) (Aznar *et al.*, 1998). In Italy, Manfredi *et al.* (1998) performed necropsies on 14 individuals

Table 1. Morphometric data, in millimeters, of *Enodiotrema megachondrus* (Looss, 1899) Looss, 1900 (Digenea: Plagiorchidae) from marine turtles (Testudines: Cheloniidae)

| Host | Looss, 1899 | Groschaft, Otero & Tenora (1977) | Santoro & Morales (2007) | Present report |
|----------------------------------|-------------|-----------------------------------|-------------------------------|----------------------------------|
| | ? | <i>E. imbricata</i> | <i>L. olivacea</i> | <i>C. mydas</i> |
| Locality | Egypt | Cuba | Costa Rica | Brazil |
| Site of infection | | Intestine | Duodeno | Small intestine |
| Number of parasites | | 27 collected (10 measurements) | 3 | 18 collected (8 measurements) |
| Body length | 5.5 | ? | 5.1 ± 0.5 (4.5 – 5.7) | 4.2 ± 0.54 (3.73 – 5.35) |
| Body width | 1*; 0.78** | ? | 1.1 ± 0.0 (1 – 1.2) | 0.879 ± 0.099 (0.761 – 1.07) |
| Oral sucker length | 0.28 | 0.185 – 0.259 | 0.200 ± 0.043 (0.150 – 0.225) | 0.183 ± 0.034 (0.112 – 0.219) |
| Oral sucker width | - | 0.222 – 0.281 | 0.242 ± 0.014 (0.225 – 0.250) | 0.212 ± 0.022 (0.192 – 0.261) |
| Pharynx length | 0.117 | - | 0.083 ± 0.014 (0.075 – 0.100) | 0.113 ± 0.014 (0.091 – 0.142) |
| Pharynx width | - | 0.074 – 0.118 | 0.108 ± 0.028 (0.075 – 0.125) | 0.124 ± 0.016 (0.105 – 0.159) |
| Ventral sucker length | 0.22 | 0.162 – 0.222 | 0.242 ± 0.014 (0.225 – 0.250) | 0.166 ± 0.034 (0.124 – 0.230) |
| Ventral sucker width | - | 0.162 – 0.244 | 0.242 ± 0.014 (0.225 – 0.250) | 0.188 ± 0.041 (0.157 – 0.278) |
| Esophagus length | 0.35 | 0.074 – 0.111 | - | 0.239 ± 0.043 (0.209 – 0.270) |
| Esophagus width | - | - | - | 0.032 ± 0.003 (30 - 35) |
| Anterior testis length | - | 0.170 – 0.222 | 0.458 ± 0.038 (0.425 – 0.500) | 0.283 ± 0.024 (0.251 – 0.324) |
| Anterior testis width | - | 0.125 – 0.222 | 0.450 ± 0.025 (0.425 – 0.475) | 0.282 ± 0.026 (0.256 – 0.331) |
| Posterior testis length | - | 0.155 – 0.214 | 0.508 ± 0.014 (0.500 – 0.525) | 0.294 ± 0.041 (0.230 – 0.347) |
| Posterior testis width | - | 0.148 – 0.199 | 0.450 ± 0.043 (0.400 – 0.475) | 0.296 ± 0.028 (0.252 – 0.344) |
| Ovary length | - | 0.096 – 0.148 | 0.250 ± 0.025 (0.225 – 0.275) | 0.193 ± 0.011 (0.178 – 0.208) |
| Ovary width | - | 0.088 – 0.148 | 0.267 ± 0.014 (0.250 – 0.275) | 0.204 ± 0.015 (0.180 – 0.224) |
| Cirrus sac length | - | 0.148 – 0.384 | - | 0.328 ± 0.033 (0.286 – 0.370) |
| Cirrus sac width | - | 0.074 – 0.222 | - | 0.207 ± 0.041 (0.176 – 0.281) |
| Mehlis`gland length | - | - | - | 0.131 ± 0.022 (0.106 – 0.165) |
| Mehlis`gland width | - | - | - | 0.128 ± 0.007 (0.119 – 0.142) |
| Eggs measurements | - | - | 10 | 20 |
| Egg length | 0.037 | 0.042 – 0.049 | 0.036 ± 0.003 (0.032 – 0.039) | 0.027 ± 0.002 (0.025 – 0.032) |
| Egg width | 0.019 | 0.019 – 0.023 | 0.014 ± 0.002 (0.013 – 0.019) | 0.012 ± 0.001 (0.009 – 0.017) |
| Right Vitelline number | 9 – 12 | 7 – 12 | - | 8 – 13 |
| Right Vitelline follicles length | | - | 0.127 ± 0.014 (0.100 – 0.150) | 0.105 ± 0.014 (0.071 – 0.149) |
| Right Vitelline follicles width | 0.15† | - | 0.102 ± 0.007 (0.100 – 0.125) | 0.92 ± 0.09 (0.071 – 0.118) |
| Left Vitelline follicles number | 9 – 12 | 7 – 12 | - | 8 - 11 |
| Left Vitelline follicles length | | - | - | 0.111 ± 0.019 (0.067 – 0.147) |
| Left Vitelline follicles width | 0.15† | - | - | 0.91 ± 0.013 (0.065 – 0.125) |

Legend: *anterior body width; ** posterior body width; †diameter

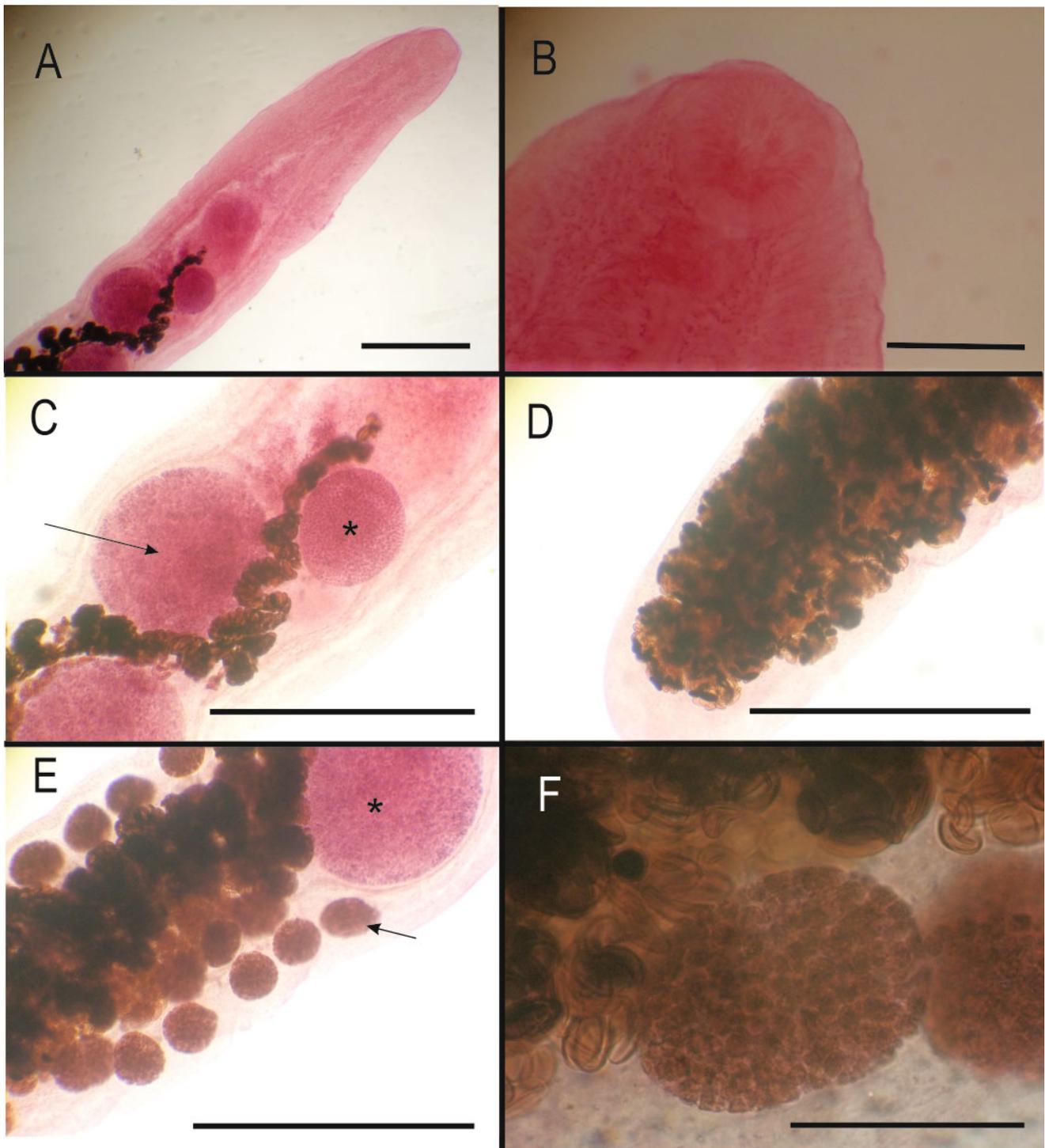


Fig. 2. *Enodiotrema megachondrus* (Looss, 1899) Looss, 1901 (Digenea: Plagiorchiidae) found in *Chelonia mydas* Linnaeus 1758 (Testudines, Cheloniidae) from Brazil (A) anterior end (scale bar= 500 µm). (B) Oral sucker (scale bar= 200 µm). (C) Ovary (asterisk) and anterior testis (arrow) (scale bar= 500 µm). (D) Posterior end and uterus (scale bar= 500 µm). (E) Viteline follicles (arrow) and posterior testis (asterisk) (scale bar= 500 µm). (F) Detail of viteline follicle and uterus with eggs (scale bar= 100 µm)

of the same species of turtle (CCL not reported) and identified *E. megachondrus* as the second most prevalent parasite, with 21.4 % collected from the stomach and intestine and an mean abundance of 32.6 (range: 0 to 392).

Santoro *et al.* (2010) studied the parasite composition in 182 individuals of *C. caretta* from six locations in the central western region of the Mediterranean Sea and found that *E. megachondrus* was the most frequent parasite, with prevalence rates ranging from 25.0 % to 96.3 % as well as mean intensity ranging from 3.0 to 131.4 (see table 2 by Santoro *et al.*, 2010).

In the archipelago of Madeira, Valente *et al.* (2009) performed a parasitological analysis of 57 individuals of *C. caretta* (CCL range: 15.3 to 61.5 cm) and the presence of *E. megachondrus* was found in 24.6 % of the hosts, accounting for the second most prevalent parasite, with mean intensity of 7.71 (range: 1 to 31).

Analyzing 70 individuals of *C. caretta* from the Adriatic Sea [mean CCL: 45.0 ± 13.6 cm (range: 25.0 to 85.4 cm)], Gracan *et al.* (2012) found a prevalence rate of 4.3 % (3/70) (confidence interval: 1.2 to 11.9) and mean intensity of 11.3 (confidence interval: 11.0 to 11.7). However, the parasites were only found in small juvenile turtles.

In *L. olivacea*, Pérez-Ponce de Leon *et al.* (1996) analyzed 32 hosts from Mexico (CCL not reported) and found *E. megachondrus* in the intestine of 43.7 % (14/32). Vivaldo *et al.* (2006) analyzed 28 hosts from the Mexican coast reported the occurrence of only seven specimens of *E. megachondrus*, with no determination of prevalence, abundance or intensity. Santoro & Morales (2007) analyzed three individuals of *L. olivacea* from Costa Rica and collected three specimens of *E. megachondrus* from the duodenum in one of the turtles analyzed.

There is only one report of the parasite in *E. imbricata* from Cuba (Groschaft *et al.*, 1977). The authors found 27 specimens of *E. megachondrus* in the intestine, but no additional information was offered.

More recently, Greiner (2013) conducted a broad survey of parasites of sea turtles found in the state of Florida (USA) between 1991 and 2006, analyzing *C. caretta*, *C. mydas*, *E. imbricata*, *D. coriacea*, *L. olivacea* and *L. kempii*. The author reports the occurrence of *E. megachondrus* in 29.5 % (13/44) of the individuals of *C. caretta* (mean intensity: 57.9 [2 to 574] with a total of 753 parasites collected from the lower intestine), in 8.1 % (6/74) of the *C. mydas* hosts (mean intensity: 13.8 [1 to 79], with a total of 85 parasites collected from the lower intestine) and 22 specimens in the upper intestine of an *L. kempii* host.

Although the life cycle of *E. megachondrus* remains unknown, Santoro *et al.* (2010) raised the hypothesis that this parasite uses pelagic intermediate hosts. This hypothesis is supported by the observation that the largest parasitological indices (prevalence, mean abundance and mean intensity) were found in turtles with small CCL during the study. A similar fact has been observed by other authors in the same host in the Mediterranean Sea (Aznar *et al.*, 1998), the Madeira archipelago (Valente *et al.*, 2009) and the Adriatic Sea (Gracan *et al.*, 2012).

The present specimen is consistent with previous descriptions, but has a smaller length, width, oral sucker, testicles, ovary and egg dimensions in comparison to those found in the literature. It was also noticed that the esophagus length, pharynx and cirrus sac were larger in comparison to information found in the literature. However, these data do not compromise the identification of the species and may merely reflect an individual variation of the specimen analyzed. Despite the slight morphometric variations, the position of the testicles, positioning of the vitelline follicles in relation to the testicles, and caeca are compatible with the species and the oral and ventral sucker relation (see Looss, 1899, 1902; Gupta & Mehrotra, 1976). In the present report, only one host were found after a beach stranding in the Brazilian region, 18 specimens of *E. megachondrus* were collected, although the occurrence of this parasite has been reported in different parts of the world (including Central America) it is the first time in the South-West Atlantic Ocean. In addition this trematode is uncommon in the Brazilian coast. Travassos *et al.* (1969) and Fernandes & Kohn (2014) through literature review does not report the *E. megachondrus* in sea turtles from Brazilian coast and the most recent analysis by Werneck and Silva (2015) and Binoti *et al.* (2016) in juvenile of *C. mydas* did not report this parasite, so the occurrence of *E. megachondrus* is an important information about the distribution of this parasite.

This note reports the first occurrence of *E. megachondrus* in *C. mydas* in the South-West Atlantic Ocean, representing a new geographic distribution for the species.

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