

## ***Helicometrina nimia* Linton, 1910 (Digenea: Opecoelidae) in dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Teleostei: Serranidae) from southeastern Brazil**

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### **Abstract**

*Helicometrina nimia* (Opecoelidae) is a digenean with wide distribution. Fish families most commonly used as hosts for *H. nimia* are Serranidae, Pomodasyidae, Scorpaenidae and Clinidae. In the present study, a new host and a new host locality are presented for the species. A description of the studied specimens, besides comments concerning its taxonomic status and biometrically compared tables of *H. nimia* reports are given. The taxonomic status of members of *Helicometrina* has been questionable. The greatest controversy for the genus seems to be related to the validity of diagnostic features, especially in regard to the number of testes. In the present study, all studied specimens presented a permanent and steady number of testes (n=9) and therefore its use as a diagnostic character is supported by the present authors. *Epinephelus marginatus* is considered a new host for *Helicometrina nimia*, and São Paulo state, southeastern Brazil, a new locality for the species.

**Keywords:** parasitology, marine fish, stomach, intestine.

## ***Helicometrina nimia* Linton, 1910 (Digenea: Opecoelidae) em garoupa verdadeira *Epinephelus marginatus* (Lowe, 1834) (Teleostei: Serranidae) no Sudeste do Brasil**

### **Resumo**

*Helicometrina nimia* (Opecoelidae) é um digenético com distribuição mundial. As famílias de peixes mais comumente utilizadas como hospedeiros por *H. nimia* são Serranidae, Pomodasyidae, Scorpaenidae e Clinidae. No presente estudo, um novo hospedeiro e uma nova localidade são apresentados para a espécie. A descrição dos espécimes estudados, além de comentários sobre o seu status taxonômico e uma tabela comparativa de medidas de *H. nimia* são apresentados. O status taxonômico dos membros de *Helicometrina* tem sido questionável. A maior controvérsia dentro do gênero parece estar relacionada com a validade das características diagnósticas, especialmente no que diz respeito ao número de testículos. No presente estudo, todos os espécimes estudados apresentaram número permanente e estável de testículos (n=9) e, portanto, sua utilização como caráter diagnóstico é suportada pelos presentes autores. *Epinephelus marginatus* é considerada um novo hospedeiro para *Helicometrina nimia* e São Paulo, sudeste do Brasil, uma nova localidade para a espécie.

**Palavras-chave:** parasitologia, peixe marinho, estômago, intestino.

### **1. Introduction**

Dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Epinephelinae) is a prominent marine fish species distributed in the warmer waters of the world including the south coast of Brazil (Atlantic ocean), Mediterranean sea, Indian ocean and southern tip of South Africa (Cribb et al., 2002). It is

a large fish species, growing up to lengths of 1 metre and weighing more than 40 kg (Smith, 1971). Epinephelinae have been cultured as an important economic alternative due to losses occasioned by diseases in shrimp culture (Rückert et al., 2009). Its parasitic fauna is highlighted

by *Helicometrina* species, of which half the species are found parasitising groupers (Cribb et al., 2002).

*Helicometrina nimia* Linton, 1910 (Digenea: Opocelidae) is a digenean with wide distribution, noticeably wider than the other species of the genus (Oliva and Muñoz, 1985). Fish families most commonly used as hosts for *H. nimia* are Serranidae, Pomodasyidae, Scorpaenidae and Clinidae (Inzunza et al., 1989).

Despite all the taxonomy controversy concerning the parasite group, the authors provide a description of the studied specimens in southeastern Brazil. Also, a new host for *H. nimia* and a comparative measurement table are presented.

## 2. Material and Methods

A total of 159 dusky grouper *E. marginatus* were collected from July 2009 to June 2010 in Ubatuba, SP, Brazil (23°26'20"S, 45°01'37"O). After being anesthetised with benzocaine, fish were sized, weighed, euthanized, and necropsied for the presence of parasites (Ethic Committee CEUA/UFSC-23080.029981/2009-76). The stomach and intestine were fixed separately (10% formalin after a hot water bath of 65°C approximately and AFA (alcohol-formalin-acetic acid), respectively) for posterior analysis by optical microscopy (OM). Digenean specimens were then washed in distilled water, stained in acetic alum carmine, diaphanised in beechwood creosote and mounted in permanent slides using Canada balsam. The ecological terms such as prevalence, mean intensity of infection and mean parasite abundance were calculated according to Bush et al. (1997). Three specimens of *H. nimia* were dissected for measurement of 22 eggs and filaments. Measurements are in millimetres, unless otherwise stated. Material was deposited in the "Helminthological Collection of Oswaldo Cruz Institute – CHIOC" under the numbers 37773 a-b.

For scanning electron microscopy (SEM) specimens were fixed in a solution of 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, and post-fixed in 2% osmium tetroxide. After washing several times in buffer, specimens were dehydrated in a graded series of alcohol, subjected to critical-point drying using liquid CO<sub>2</sub> and examined using a scanning electron microscope (Zeiss DSM-940A) at EMBRAPA (Pelotas City, RS, Brazil).

For histological sections specimens of *H. nimia* previously fixed in 10% buffered formalin, were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. The sections (3-7µm) were then stained with standard Harris's haematoxylin and eosin (HH and E) stain. Permanent microscopy slides were prepared in Erv-mount.

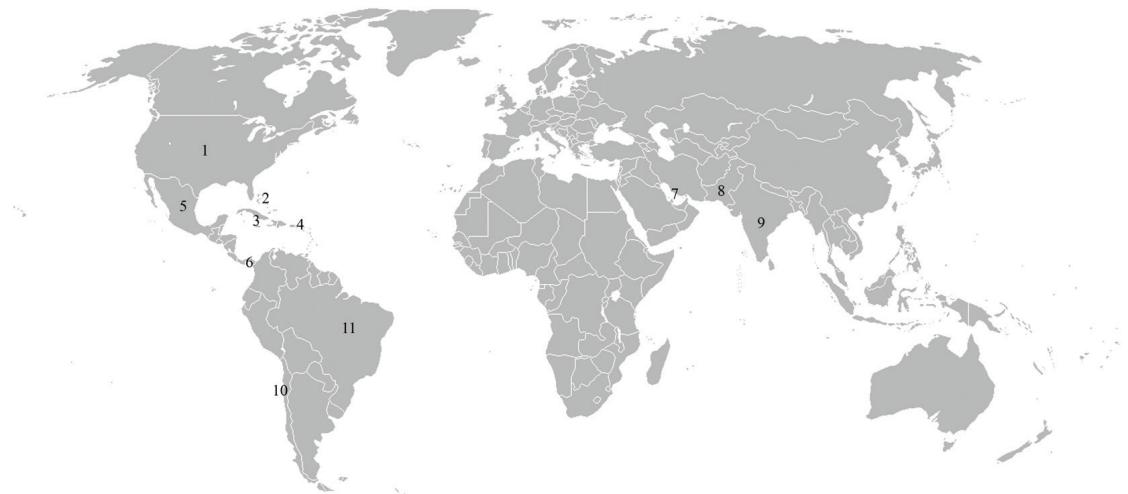
## 3. Results

Description of *Helicometrina nimia* Linton, 1910

Taxonomic summary

Synonyms: *Helicometrina orientalis* (Srivastava, 1936) and *Helicometrina elongata* (Hardy, 1972) (by Deelman, 1960, not accepted by Saoud et al., 1988).

Hosts: *Lutjanus griseus* (Linnaeus, 1758) (= *Neomaensis griseus*), *Lutjanus guttatus* (Steindachner, 1869) (= *Neomaensis guttatus*), *Ocyurus chrysurus* (Bloch, 1791), *Calamus calamus* (Valenciennes, 1830), *Stegastes leucostictus* (Müller and Troschel, 1848) (= *Eupomacentrus leucostictus*), *Opsanus beta* (Goode and Bean, 1880), *Scorpaena grandicornis* Cuvier, 1829 (Type hosts, Linton, 1910); *L. guttatus* (= *N. guttatus*) (Bravo, 1954); *Cephalopholis fulva* (Linnaeus, 1758) (Sparks, 1957; Caballero, 1990; Nahhas and Carlson, 1994); *Epinephelus analogus* Gill, 1863 (Sogandares-Bernal, 1959); *Scorpaena plumieri* Bloch, 1789 (Siddiqi and Cable, 1960; Travassos et al., 1967); *Mycteroperca rosacea* (Streets, 1877) (Arai, 1962); *Haemulon sciurus* (Shaw, 1803),



**Figure 1.** World distribution of *Helicometrina nimia*. 1. United States; 2. Bahamas; 3. Jamaica; 4. Puerto Rico; 5. Mexico; 6. Panama; 7. Arabian Gulf; 8. Pakistan; 9. India; 10. Chile; 11. Brazil. Source: adapted from [http://pt.wikipedia.org/wiki/Ficheiro:Mapa\\_Mundi\\_Detalle\\_Max\\_2008.png](http://pt.wikipedia.org/wiki/Ficheiro:Mapa_Mundi_Detalle_Max_2008.png)

*Hypoplectrus indigo* (Poey, 1851), *Lachnolaimus maximus* (Walbaum, 1792), *Lutjanus jocu* (Bloch and Schneider, 1801), *Bothus lunatus* (= *Platophrys lunatus*) (Linnaeus, 1758), *Sphaeroides spengleri* (Bloch, 1785) (Nahhas and Cable, 1964); *Lagocephalus laevis* (Linnaeus, 1766) (Travassos et al., 1967); *Epinephelus diacanthus* (Valenciennes, 1828) (Zaidi and Khan, 1977; Bilqees, 1981); *Nemipterus peronii* (= *N. tolu*) (Valenciennes, 1830) (Al-Yamani and Nahhas, 1981); *Cymatogaster aggregata* Gibbons, 1854 (Arai et al., 1988); *Calliclinus geniguttatus* (Valenciennes, 1836), *Calliclinus nudiventris* Cervigón and Pequeño, 1979 (Inzunza et al., 1989); *Paralabrax humeralis* (Valenciennes, 1828), *Acanthistius pictus* (Tschudi, 1846), *Anisotremus scapularis* (Tschudi, 1846) (Oliva and Muñoz, 1985); *Sebastes capensis* (Gmelin, 1789) (Oliva and Muñoz, 1985; Bray, 2001; Oliva and Gonzalez, 2004); *Cephalopholis fulvus* (Linnaeus, 1758) (Caballero, 1990); *Bothus lunatus* (Linnaeus, 1758) (León, 1992); *Hemilutjanus macrophthalmos* (Tschudi, 1846), *Cheilodactylus variegatus* Valenciennes, 1833, *Genypterus maculatus* (Tschudi, 1846) and *Mugiloides chilensis* (Molina, 1782) (Luque and Oliva, 1993); *Lutjanus synagris* (Linnaeus, 1758), *Conodon nobilis* (Linnaeus, 1758), *Umbrina broussoneti* (Cuvier, 1830) (Bunkley-Williams et al., 1996); *Sebastes nebulosus* Ayres, 1854 (Gibson, 1996); *Epinephelus morio* (Valenciennes, 1828) (Moravec et al., 1997); *Polydactylus sextarius* (Bloch and Schneider, 1801) (Gudivada and Vankara, 2010); *Scartichthys viridis* (Valenciennes, 1836) (Muñoz-Muga and Muñoz, 2010); *Epinephelus marginatus* (present study).

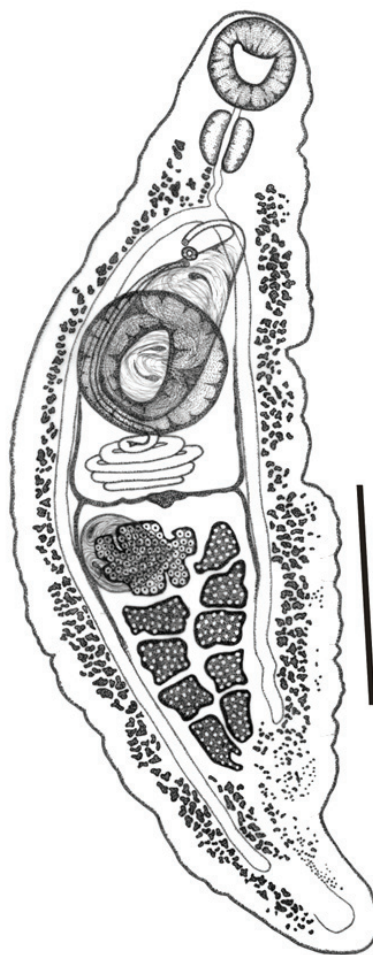
Travassos et al. (1967) reported other hosts for *H. nimia* such as *Amphistichus argenteus* Agassiz, 1854; *Apogon maculatus* (Poey, 1860); *Apogon pseudomaculatus* Longley, 1932; *Balistes vetula* Linnaeus, 1758; *Clinocottus analis* (Girard, 1858); *Coryphaena hippurus* Linnaeus, 1758; *Embiotoca jacksoni* Agassiz, 1853; *Haemulon album* Cuvier, 1830; *Leptocottus armatus* Girard, 1854; *Lutjanus apodus* (Walbaum, 1792); *Menticirrhus undulatus* (Girard, 1854); *Opisthognathus maxillosus* Poey, 1860; *Paralabrax clathratus* (Girard, 1854); *Paralabrax maculatofasciatus* (Steindachner, 1868); *Paralabrax nebulifer* (Girard, 1854); *Scorpaena agassizii* Goode and Bean, 1896; *Scorpaena brasiliensis* Cuvier, 1829; *Scorpaena sonorae* Jenkins and Evermann, 1889; *Scorpaenichthys marmoratus* (Ayres, 1854); *Sebastes atrovirens* (Jordan and Gilbert, 1880); *Sebastes serriceps* (Jordan and Gilbert, 1880); *Syacium papillosum* (Linnaeus, 1758); *Synodus lucioceps* (Ayres, 1855); *Xenistius californiensis* (Steindachner, 1876).

**Localities** (Figure 1): Tortugas, United States (Type locality) Linton, 1910; United States (Arai et al., 1988); Bahamas (Sparks, 1957; Sogandares-Bernal and Hutton, 1959); Jamaica (Nahhas and Carlson, 1994); Puerto Rico (Siddiqi and Cable, 1960; Bunkley-Williams et al., 1996); Mexico (Manter, 1940; Bravo, 1954; Arai, 1962; Caballero, 1990; León, 1992; Moravec et al., 1997); Panama (Sogandares-Bernal and Hutton, 1959); Arabian Gulf (Al-Yamani and Nahhas, 1981); Pakistan (Bilqees, 1981); India (Gudivada and Vankara, 2010); Chile (Oliva

and Muñoz, 1985; Inzunza et al., 1989; Luque and Oliva, 1993; Oliva and Gonzalez, 2004; Muñoz-Muga and Muñoz, 2010); Brazil (Travassos et al., 1967; present study).

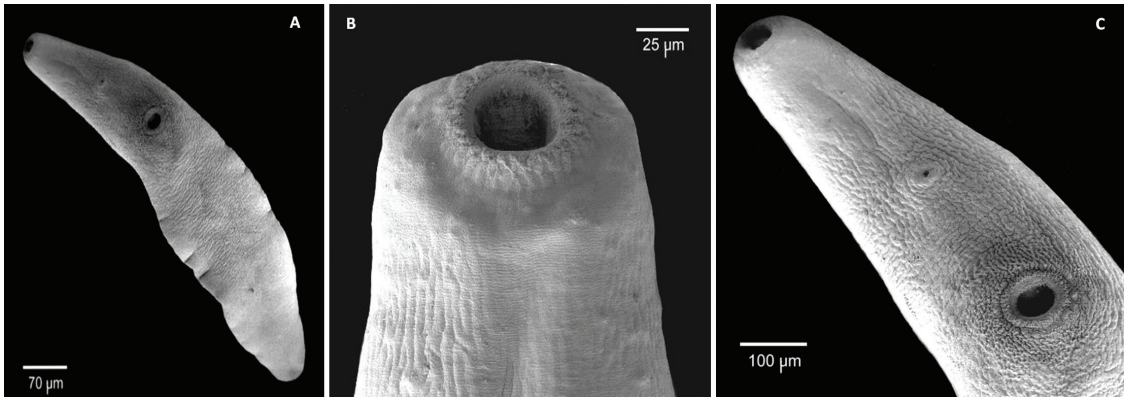
**Prevalence, mean intensity of infection, abundance and site of infection:** *Helicometrina nimia* was observed in 19.49% of total examined fish. The mean abundance was  $1.19 \pm 4.02$  and mean intensity of infection of  $6.13 \pm 7.33$ . In 68.42% of parasitised fish, *H. nimia* was found in intestine and 31.58% in stomach.

**Description of the studied specimens** (based in 23 specimens; 16 mature; 7 young) (Figure 2): Leaf-shaped body,  $1.450 \pm 0.471$  (0.680-2.240) length and  $0.444 \pm 0.177$  (0.240-1.040) greatest width. Tegument rugose. Roughness of the tegumental surface surrounding oral and ventral sucker visible under SEM (Figures 3A-C). Oral sucker subterminal, circular, muscular,  $0.135 \pm 0.037$  (0.088-0.224) length and  $0.135 \pm 0.029$  (0.088-0.200) width. Pharynx muscular,  $0.086 \pm 0.018$  (0.056-0.128) length and  $0.083 \pm 0.019$  (0.040-0.128) width. Esophagus long,  $0.091 \pm 0.042$  (0.024-0.160) length. Bifurcation of the intestinal caeca anterior to acetabulum. Caeca long,

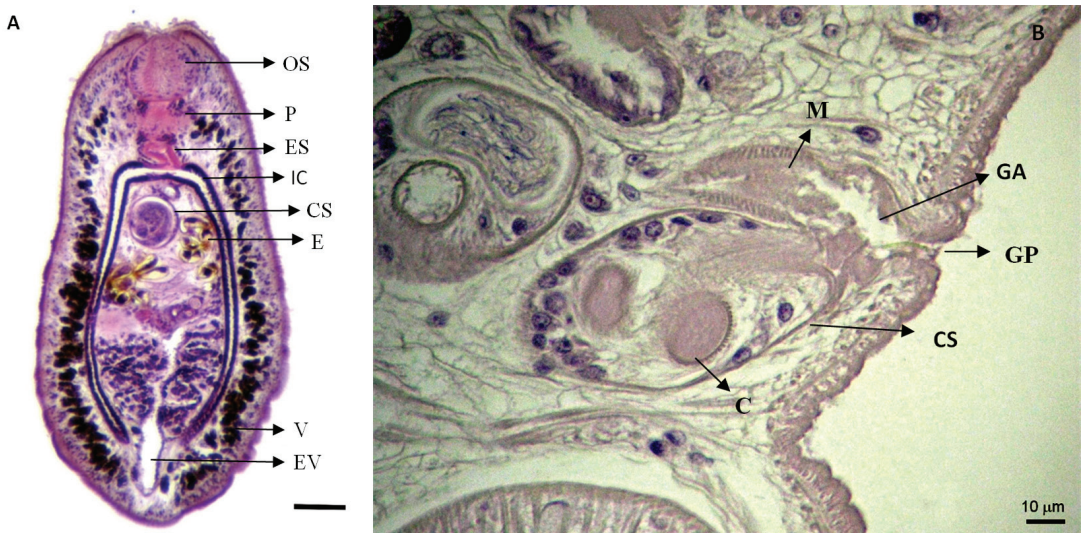


**Figure 2.** *Helicometrina nimia* Linton, 1910 from southeastern Brazil. Ventral view. Bar: 8.5mm.





**Figure 3.** Scanning electron micrographs (SEM) of *Helicometrina nimia*. A. Whole mount (ventral view); Bar: 70 mm. B. Anterior end showing oral sucker; Bar: 25 mm. C. Anterior extremity showing oral sucker, genital pore and ventral sucker; Bar: 100 mm.



**Figure 4.** Histological sections of *Helicometrina nimia*. A. Whole mount: oral sucker (OS); pharynx (P); esophagus (ES); intestinal caecum (IC); cirrus sac (CS); egg (E); vitellaria (V); testes (T); excretory vesicle (EV). HH and E; Bar: 100 mm. B. Common genital atrium shared with metraterm and cirrus sac. Genital pore found within genital atrium. Metraterm (M); genital atrium (GA); genital pore (GP); cirrus sac (CS); cirrus (C). HH and E; Bar: 10 mm.

terminating blindly, extending to near posterior body end. Acetabulum round, muscular, larger than oral sucker with  $0.222 \pm 0.051$  (0.136-0.360) length and  $0.215 \pm 0.053$  (0.136-0.296). Genital atrium anterior to acetabulum. Genital pore posterior to bifurcation,  $0.466 \pm 0.074$  (0.376-0.544) away from anterior end of body. Cirrus sac large  $0.159 \pm 0.084$  (0.056-0.280) extending to posterior margin of acetabulum in mature specimens or to its equator in younger specimens. Seminal receptacle superimposed on the ovary with  $0.141 \pm 0.041$  (0.096-0.176) length and  $0.157 \pm 0.036$  (0.120-0.192) width. Nine testes in two intercecal rows (four in the right row and five in the left row), with irregular shapes and  $0.074 \pm 0.026$  (0.024-0.128) length and  $0.089 \pm 0.032$  (0.024-0.168) width. Spiral uterus with many coils extending next to equator. Ovary with 4-6 lobes between uterus and anterior testes, with  $0.126 \pm 0.044$  (0.064-0.192) length and  $0.160 \pm 0.050$  (0.072-0.240) width. Vitellaria consisting of numerous irregularly shaped,

densely packed follicles forming 2 extra-cecal lateral fields connected anteriorly between esophagus and cirrus sac and posteriorly in post-testicular region. Sinistral and dextral common vitelline ducts merge to form transverse median vitelline reservoir situated between uterus and ovary (Figures 4A-B). Oval egg averaging  $73 \text{ mm} \pm 0.008$  (60-90) long by  $30 \pm 0.006 \text{ mm}$  (20-40) wide with a long unipolar filament with  $0.554 \pm 0.119$  (0.430-0.830) long or approximately 7.6 times length of capsule. Excretory vesicle long, median, with  $0.615 \pm 0.283$  (0.216-1.056) length extending from posterior limit of ovary to posterior body extremity. Excretory pore dorso-terminal.

#### 4. Discussion

*Helicometrina nimia* was first described by Linton (1910) from marine fishes of Tortugas, Florida and it is currently distributed in almost the entire American continent

Table 1. Comparative measurements of *Helicometrina nimia* Linton, 1910. The average  $\pm$  standard deviation is followed by minimum and maximum values in parenthesis.

| Hosts                     | Present Study  | Linton (1910)   |  | Travassos et al. (1967)   |   | Oliva and Muñoz (1985)   |  | Inzunza et al. (1989) |           | Luque and Oliva (1993) |           | Moravec et al. (1997) |                          |
|---------------------------|--|---|--|---|---|--|--|-----------------------|-----------|------------------------|-----------|-----------------------|--------------------------|
|                           |  | <i>Luijanus griseus</i> , <i>L. guttatus</i> , <i>Ocyurus chrysurus</i> , <i>Calamius calamus</i> , <i>Stegastes leucostictus</i> , <i>Opsanus beta</i> , <i>Scorpaena grandicornis</i> | <i>Scorpaena plumieri</i>                            | <i>Sebastes capensis</i> , <i>Paralabrax humeralis</i> , <i>Acanthissius pictus</i> , <i>Anisotremus scapularis</i> | <i>Calliclinus geniguttatus</i> , <i>Calliclinus nudiventris</i>  | <i>Hemilutjanus macrophthalmus</i> , <i>Cheilodactylus variegatus</i> , <i>Mudiloides dulensis</i> | <i>Epinephelus marginatus</i>                        | Stomach and intestine | Intestine | Intestine              | Intestine | Intestine             | <i>Epinephelus morio</i> |
| Site of infection         |  |   |  |   |   |  |  |                       |           |                        |           |                       |                          |
| Body                      | 1.450 (0.680-2.240) <sup>L</sup><br>0.444 (0.240-1.040) <sup>W</sup> | 1.04-2.46 <sup>L</sup><br>0.38-0.91 <sup>W</sup>  | 3.76-4.26 <sup>L</sup><br>1.49-1.68 <sup>W</sup>     | 5.31 <sup>L</sup><br>1.56 <sup>W</sup>  | 0.86 (0.64-1.10) <sup>L</sup><br>0.46 (0.34-0.64) <sup>W</sup>    | 2.02 (1.65-2.30) <sup>L</sup><br>0.58 (0.40-0.64) <sup>W</sup>                                     | 0.870-0.992 <sup>L</sup><br>0.286 <sup>W</sup>       |                       |           |                        |           |                       |                          |
| Oral sucker               | 0.135 (0.088-0.224) <sup>L</sup><br>0.135 (0.088-0.200) <sup>W</sup> | 0.1-0.15 <sup>D</sup>   | 0.27-0.32 <sup>L</sup><br>0.28-0.30 <sup>W</sup>     | 0.30 <sup>L</sup><br>0.28 <sup>W</sup>  | 0.12 (0.10-0.14) <sup>L</sup><br>0.13 (0.10-0.16) <sup>W</sup>    | 0.14 (0.11-0.16) <sup>D</sup>  | 0.090-0.111 <sup>L</sup><br>0.105-0.117 <sup>W</sup> |                       |           |                        |           |                       |                          |
| Pharynx                   | 0.086 (0.056-0.128) <sup>L</sup><br>0.083 (0.040-0.128) <sup>W</sup> | 0.06-0.10 <sup>L</sup><br>0.55-0.08 <sup>W</sup>  | 0.117-0.144 <sup>L</sup><br>0.150-0.183 <sup>W</sup> | 0.17 <sup>L</sup><br>0.15 <sup>W</sup>  | 0.07 (0.06-0.09) <sup>L</sup><br>0.06 (0.06-0.08) <sup>W</sup>    | -  | -  |                       |           |                        |           |                       |                          |
| Oesophagus                | 0.091 (0.024-0.160) <sup>L</sup>                                     | -   | -  | -   | -   | -  | -  |                       |           |                        |           |                       |                          |
| Acetabulum                | 0.222 (0.136-0.360) <sup>L</sup><br>0.215 (0.136-0.296) <sup>W</sup> | 0.17-0.29 <sup>D</sup>  | 0.45-0.48 <sup>D</sup>                               | 0.5 <sup>D</sup>  | 0.23 (0.18-0.29) <sup>L</sup><br>0.24 (0.19-0.30) <sup>W</sup>    | 0.20 (0.18-0.21) <sup>D</sup>  | 0.165-0.174 <sup>L</sup><br>0.156-0.195 <sup>W</sup> |                       |           |                        |           |                       |                          |
| Genital pore <sup>A</sup> | 0.466 (0.376-0.544) <sup>L(A)</sup>                                  | -   | -  | -   | -   | -  | -  |                       |           |                        |           |                       |                          |
| Ovary                     | 0.126(0.064-0.192) <sup>L</sup><br>0.160 (0.072-0.240) <sup>W</sup>  | -   | 0.22-0.27 <sup>L</sup><br>0.52-0.55 <sup>W</sup>     | 0.54 <sup>L</sup><br>0.33 <sup>W</sup>  | 0.15 (0.10-0.24) <sup>L</sup>                                     | -  | 0.033-0.036 <sup>L</sup> 0.045-0.051 <sup>W</sup>    |                       |           |                        |           |                       |                          |
| Testes                    | 0.074 (0.024-0.128) <sup>L</sup><br>0.089 (0.024-0.168) <sup>W</sup> | -   | 0.24-0.29 <sup>L</sup><br>0.37-0.43 <sup>W(B)</sup>  | 0.35 <sup>D</sup>   | 0.95 (0.05-0.13) <sup>L</sup><br>0.05 (0.02-0.09) <sup>W(B)</sup> | -  | 0.033-0.048 <sup>L</sup> 0.027-0.051 <sup>W</sup>    |                       |           |                        |           |                       |                          |
| Cirrus sac                | 0.159 (0.056-0.280) <sup>L</sup>                                     | -   | 0.73-0.83 <sup>L</sup><br>0.23-0.25 <sup>W</sup>     | 1.03 <sup>L</sup><br>0.31 <sup>W</sup>  | -   | -  | 0.102-0.120 <sup>L</sup> 0.027-0.039 <sup>W</sup>    |                       |           |                        |           |                       |                          |
| Seminal receptacle        | 0.141 (0.096-0.176) <sup>L</sup><br>0.157 (0.120-0.192) <sup>W</sup> | -   | 0.13-0.16 <sup>L</sup><br>0.56-0.64 <sup>W</sup>     | -   | -   | -  | -  |                       |           |                        |           |                       |                          |
| Excretory vesicle         | 0.615 (0.216-1.056) <sup>L</sup>                                     | -   | -  | -   | -   | -  | -  |                       |           |                        |           |                       |                          |
| Egg (mm)                  | 73 (60-90) <sup>L</sup><br>30 (20-40) <sup>W</sup>                   | 50 <sup>L</sup><br>30 <sup>W</sup>  | 66 <sup>L</sup><br>26 <sup>W</sup>                   | 56 <sup>L</sup>   | 49.6 (42.8-61.9) <sup>L</sup><br>22.9 (19.0-28.5) <sup>W</sup>    | 58 <sup>L</sup><br>30 <sup>W</sup>   | -  |                       |           |                        |           |                       |                          |

<sup>L</sup>Length, <sup>W</sup>width, <sup>D</sup>diameter, <sup>A</sup> distance from the anterior extremity; <sup>B</sup> average of the testes on the right and the left side.

(in many fish families), besides reports from the Arabian Gulf, Pakistan and India (Al-Yamani and Nahhas, 1981; Bilqeas, 1981; Gudivada and Vankara, 2010).

According to Mittal and Pande (2007), the valid species of *Helicometrina* are *H. nimia*; *H. parva* Manter; *H. septorchis* Srivastava; *H. mirzai* Siddiqi and Cable; *H. quadrorchis* Manter and Pritchard; *H. hexorchis* Gupta and Sehgal; *H. scomberi* Gupta and Jahan; *H. unicum* Gupta and Puri and *H. chauhani* Mittal and Pande. The authors also presented a key to the species of genus based on the number of testes.

Saoud et al. (1988) described *H. qatarensis* (not included in the valid list species proposed by Mittal and Pande (2007) and differentiated this species from *H. nimia* mainly in the position of the genital pore and the larger eggs. The authors did not recognise the synonymisation of *H. nimia* and *H. orientalis* proposed by Deelman (1960) since these species present a different arrangement of the vitellaria distribution, according to the former authors.

The taxonomic status of members of *Helicometrina* has been questionable. The greatest controversy for the genus seems to be related to the validity of diagnostic features, especially in regard to the number of testes. Hafeezullah (1971) studied 28 specimens of *Helicometrina* from unrelated species of marine fishes from India with varying number of testes. Except for this characteristic, the author did not report any other difference among specimens and concluded that the number of testes should not form a character to distinguish species. Later, Gibson et al. (2005) recognized the logic of the comments of the later author, but supported a distinction on the number of testes. In the present study, all studied specimens presented a permanent and steady number of testes (n=9) and therefore its use as a diagnostic characteristic is supported by the present authors. Moreover, the arrangement of testes also remained the same in all analysed specimens, distributed in two rows, with four testes on the right side and five on the left. However, differently from the above studied specimens previous reports for *H. nimia* presented an inverted arrangement of the row of testicles (Oliva and Muñoz, 1985; León, 1992; Bunkley-Williams et al., 1996; Moravec et al., 1997). Even though the arrangement of testes in the present studied specimens (4 testes in the right row and 5 in the left row) was horizontally inverted when compared to previous reports for the species, the authors do not consider that this difference might support a distinct species of *H. nimia*. Linton (1910) observed that in the specimens having testes, the cells were “loosely clustered” and appeared disintegrating. Manter (1933) attributed it to the “weakness of the male gonads”. Such a characteristic was not perceived in mature specimens in the present study, but it was possible to note in younger individuals the presence of testes not as compact as in the mature ones, giving the impression mentioned by both authors.

Measurements of the present studied specimens agree with previous descriptions of *H. nimia* such as those provided by Linton (1910), Inzunza et al. (1989), Luque and Oliva (1993) and Moravec et al. (1997) (Table 1).

Other features pointed out by Hafeezullah (1971) are, in fact, highly variable and should not be used for species distinction. The extent of the cirrus sac seems to be related with the parasite age. It was observed that mature specimens presented larger cirrus sac than the younger ones. It was commonly observed a fold in the anterior part of the cirrus sac (as showed in the drawings of many other previous reports), which may be responsible for a decrease in its length.

Bunkley-Williams et al. (1996) reported paddle-shaped expansions in the extremity of each caeca of *H. nimia*. Differently from the later, in the present study such a characteristic was not observed in any studied specimens.

With respect to the ovary, the authors agree with the remarks of Oliva and Muñoz (1985) since it was also observed a great variability both in shape and number of ovary's lobes, being noticed, as the only pattern, the existence of four primary lobes, which may present a varied secondary lobulation (4-6).

For all anteriorly mentioned, the authors believe that the number of testes, the position of the genital pore, the egg size, vitellaria arrangement and extension, the position and shape of the ventral sucker as well as the pharynx size are good indicators for *Helicometrina* species distinction. In this way, specimens analysed in the present study are considered *H. nimia*.

In the present study, *Epinephelus marginatus* is considered a new host for *Helicometrina nimia*, and São Paulo state, southeastern Brazil, can be considered a new locality for the species.

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