Part I

SURVEY, POPULATION DYNAMICS AND DESCRIPTION OF

*DINURUS* SPECIES FROM *CORYPHAENA HIPPURUS*
Chapter 1

SURVEY AND POPULATION DYNAMICS

INTRODUCTION

An ecological approach to problems in Parasitology is often neglected because most of the works are confined to descriptions and lists of parasites and to the clinical and economical considerations. Since parasitic infections are usually influenced by extrinsic and intrinsic factors associated with definitive and intermediate hosts, it is felt that through such an approach, parasitologists get an opportunity to extend the range of their contributions to an understanding of the basic problems in biology of the parasites. Review of the literature on Dinurus reveals that no work has been done on the ecology and population dynamics of these parasites. This, it seems, may be because of the difficulties in obtaining the host fishes at definite time intervals and study them under their natural conditions. Most of the present day information regarding the survey and taxonomy of the Dinurus by leading workers are from preserved host animals collected from far off places. Kerala, the Southern part of India, experiences clear cut seasonal variations and host animals are available in all seasons of the year. Therefore it was thought desirable to study the ecology and population dynamics of the Dinurus species of Kerala.

Looss (1899, 1907 & 1908) reported the occurrence of Dinurus and other hemiurids from Egyptian seas. Studies on the helminth fauna of Japan including Dinurus were carried out by Yamaguti (1934, 1937, 1938, 1940, 1942, 1951 &
Flukes of British fishes were studied by Dawes (1947) and of Miami region were recorded by Ward (1954). Carbonell et al. (1999) reported *Dinurus tornatus*, *D. breviductus*, and *D. longisinus* from *C. hippurus*. Except for these studies nothing is recorded about the occurrence, distribution and population dynamics of *Dinurus* species parasitizing the dolphin fishes.

Nine species of the genus *Dinurus* have been reported so far. They are *D. tornatus* Rudolphi, 1819; *D. barbatus* Cohn, 1902; *D. breviductus* Looss, 1907; *D. longisinus* Looss, 1907; *D. coryphaena* Yamaguti, 1934; *D. euthynni* Yamaguti, 1934; *D. scombri* Yamaguti, 1934; *D. hippuri* Nadakal et al., 1991 and *D. ivanosi* Rekha & John, 2004. *Coryphaena equisetis*, *C. hippurus*, *Euthynnus alletteratus*, *E. pelamys*, *Pelamys sarda*, *Peorilus paru*, *Scomber japonicus*, *Seriola aurevittata*, and *S. lalandi* were the host fishes reported. *D. scombri* (*D. selari* Parukhin, 1966) and *D. euthynni* were reported from *S. japonicus* and *E. pelamys* respectively (Yamaguti, 1971). As part of the studies on *Dinurus* a survey of these flukes were made in *C. hippurus* collected from different localities in Kerala. This study is therefore an attempt to contribute to the seasonal distribution and host record of *Dinurus* occurring in India, particularly Kerala.

**MATERIALS AND METHODS**

Fresh specimens of *C. hippurus* were collected from different fish landing centers in Kerala. As many as 2204 hosts were collected for the present study. Separated the stomach, exposed and examined for parasites. For extensive number of worms, whole gut contents rinsed out into beakers containing saline mixed with
sodium bicarbonate (one spoonful per liter) to remove mucus, and allowed parasites to settle. The different Dinurus species and their microhabitat in the host were recorded on data sheet. Sorted out worms, transferred to physiological saline (0.6%) and fixed in different fixatives (mentioned in Table 6) for taxonomic, anatomic, histological and histochemical works. Taxonomic observations were made on both live and fixed specimens. Data obtained were analyzed species wise for biostatistical parameters such as incidence (% of infection) and intensity (total worm burden/number of hosts infected). The host animals dissected, number infected and the number and species of Dinurus recovered for a period of consecutive 4 years (from January 2000 to December 2003) are listed in Tables 1-5.

**OBSERVATIONS**

Overall incidence of Dinurus in C. hippurus was 97.01% in 2000, 99.03% in 2001, 95.88% in 2002 and 96.65% in 2003. Of the 2,202 hosts examined, only 68 were found uninfected (Table 1). A total of 3, 27,149 worms were recovered from 2134 fishes infected. Eight species of Dinurus recovered during the study, were D. hippuri, D. longisinus, D. tornatus, D. barbatus, D. scombri, D. breviductus, D. coryphaenae and D. ivanosi. Although many hosts were infected with more than one species of Dinurus, those having a combination of D. tornatus, D. hippuri and D. longisinus were common. Hosts with single species were rare. Hosts collected during southwest monsoon were characterized by juvenile Dinurus. The intensity of infection was high during monsoon season.
D. tornatus, D. hippuri, D. barbatus and D. longisinus were recovered in all the four seasons of the period of study (Figs. 4 - 7). The most abundant species reported was D. hippuri. The incidence of infection of D. hippuri was low during north-east monsoon and winter in all the four annual cycles. In each annual cycle, there were two peaks of incidence; in summer and south-west monsoon. Incidence of infection of D. longisinus also showed a more or less similar pattern. The intensity of infection of D. tornatus was high during monsoon in all the four reported years. In 2000 and 2001 higher incidence of D. tornatus infection was observed in south-west monsoon, while in 2002 and 2003 during north-east monsoon. D. tornatus infection was low in winter and summer in four annual cycles. Though D. barbatus was recovered in all seasons, the intensity of infection was low compared to D. hippuri, D. longisinus and D. tornatus.

D. ivanosi and D. scombri were recovered from 2002 and 2003 collections. D. ivanosi was mostly encountered in winter and summer, while D. scombri only in south-west monsoon. The number of D. scombri collected in 2002 and 2003 were 53 and 23 respectively. The total number of D. ivanosi collected in two annual cycles was 24. D. coryphaenae and D. breviductus were recovered during southwest and northeast monsoons of two annual cycles.

In the season wise analysis it was found that the incidence of D. tornatus infection was generally high in the rainy seasons, while that of D. hippuri, D. longisinus and D. barbatus high in the summer and south-west monsoon. D. breviductus, D. scombri, D. coryphaenae and D. ivanosi had no definite seasonal pattern.
Table 1

Data on host fish dissected, number infected and *Dinurus* recovered

<table>
<thead>
<tr>
<th>Year</th>
<th>Number dissected</th>
<th>Number infected</th>
<th>Percentage infected (incidence)</th>
<th><em>Dinurus</em> recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>570</td>
<td>553</td>
<td>97.01</td>
<td>D. hippuri, D. longisinus, D. tornatus, D. barbatus</td>
</tr>
<tr>
<td>2002</td>
<td>486</td>
<td>466</td>
<td>95.88</td>
<td>D. hippuri, D. longisinus, D. tornatus, D. barbatus, D. scombri, A new species later named <em>D. ivanosi</em></td>
</tr>
</tbody>
</table>
### Table 2
Season wise data on *Dinurus* from *C. hippocus* in 2000

<table>
<thead>
<tr>
<th>Season</th>
<th>Different species and their number</th>
<th>Incidence</th>
<th>Dh</th>
<th>Dl</th>
<th>Dt</th>
<th>Db</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan-Feb</td>
<td></td>
<td>94.29</td>
<td>8735</td>
<td>4543</td>
<td>2456</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(64.7)*</td>
<td>(33.7)</td>
<td>(18.2)</td>
<td>(1.39)</td>
</tr>
<tr>
<td>Summer</td>
<td></td>
<td>96.48</td>
<td>15321</td>
<td>8933</td>
<td>6431</td>
<td>345</td>
</tr>
<tr>
<td>Mar-May</td>
<td></td>
<td></td>
<td>(110)</td>
<td>(64.2)</td>
<td>(46.3)</td>
<td>(2.48)</td>
</tr>
<tr>
<td>S-W M.</td>
<td></td>
<td>100</td>
<td>18432</td>
<td>16298</td>
<td>11672</td>
<td>160</td>
</tr>
<tr>
<td>May-Sept</td>
<td></td>
<td></td>
<td>(130)</td>
<td>(115)</td>
<td>(82.8)</td>
<td>(1.13)</td>
</tr>
<tr>
<td>N-E M.</td>
<td></td>
<td>97.23</td>
<td>6124</td>
<td>2283</td>
<td>9718</td>
<td>66</td>
</tr>
<tr>
<td>Oct-Dec</td>
<td></td>
<td></td>
<td>(44.4)</td>
<td>(16.5)</td>
<td>(70.4)</td>
<td>(0.47)</td>
</tr>
<tr>
<td>Whole year</td>
<td></td>
<td>97.01</td>
<td>48,612</td>
<td>32,057</td>
<td>30,277</td>
<td>759</td>
</tr>
<tr>
<td>of study</td>
<td></td>
<td></td>
<td>(87.9)</td>
<td>(58.0)</td>
<td>(54.8)</td>
<td>(1.37)</td>
</tr>
</tbody>
</table>

* Figures in parenthesis indicate intensity of infection

[Dh: Dinurus barbatus, Dh: D. hippocus, Dl: D. longisinus, Dt: D. tornatus,
N-E M: Northeast monsoon, S-W M: Southwest monsoon]
Fig. 4

Graphical representation of *Dinurus* collected in 2000

N-E: northeast, S-W: southwest]
Table 3

Season wise data on *Dinurus* from *C. hippurus* in 2001

<table>
<thead>
<tr>
<th>Season</th>
<th>Different species and their number</th>
<th>Incidence</th>
<th><em>Dh</em></th>
<th><em>Dl</em></th>
<th><em>Dt</em></th>
<th><em>Db</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter Jan-Feb</td>
<td></td>
<td>98.57</td>
<td>4821</td>
<td>2991</td>
<td>1567</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(63.4)*</td>
<td>(39.4)</td>
<td>(20.6)</td>
<td>(1.47)</td>
<td></td>
</tr>
<tr>
<td>Summer Mar-May</td>
<td></td>
<td>100</td>
<td>9811</td>
<td>3456</td>
<td>2182</td>
<td>225</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(127)</td>
<td>(44.9)</td>
<td>(28.3)</td>
<td>(2.92)</td>
<td></td>
</tr>
<tr>
<td>S-W M. May-Sept</td>
<td></td>
<td>100</td>
<td>19165</td>
<td>13768</td>
<td>1596</td>
<td>532</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(245)</td>
<td>(176)</td>
<td>(20.5)</td>
<td>(6.82)</td>
<td></td>
</tr>
<tr>
<td>N-E M. Oct-Dec</td>
<td></td>
<td>97.38</td>
<td>4420</td>
<td>2879</td>
<td>7312</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(58.2)</td>
<td>(37.8)</td>
<td>(96.2)</td>
<td>(1.22)</td>
<td></td>
</tr>
<tr>
<td>Whole year of study</td>
<td></td>
<td>97.03</td>
<td>38,217</td>
<td>23,094</td>
<td>12,657</td>
<td>962</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(124)</td>
<td>(75.2)</td>
<td>(41.2)</td>
<td>(3.13)</td>
<td></td>
</tr>
</tbody>
</table>

* Figures in parenthesis indicate intensity of infection

[*Db: Dinurus barbatus, Dh: D. hippuri, Dl: D. longisinus, Dt: D. tornatus, N-E M- Northeast monsoon, S-W M- Southwest monsoon*]
Fig. 5

Graphical representation of Dinurus collected in 2001

[Db: Dinurus barbatus, Dh: D. hippuri, Dl: D. longisinus, Dt: D. tomatus

N-E northeast, S-W: southwest]
Table 4

Season wise data on Dinurus from C. hippurus in 2002

<table>
<thead>
<tr>
<th>Season</th>
<th>Incidence</th>
<th>Dh</th>
<th>Dl</th>
<th>Dt</th>
<th>Db</th>
<th>Ds</th>
<th>Di</th>
<th>Dc</th>
<th>Dbr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan-Feb</td>
<td>93.22</td>
<td>2290</td>
<td>1477</td>
<td>1909</td>
<td>112</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(13.8)</td>
<td>(8.9)</td>
<td>(11)</td>
<td>(0.7)</td>
<td></td>
<td>(0.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>95.83</td>
<td>8821</td>
<td>3688</td>
<td>1325</td>
<td>239</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mar-May</td>
<td></td>
<td>(46.9)</td>
<td>(31.3)</td>
<td>(7.1)</td>
<td>(1.3)</td>
<td></td>
<td>(0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-W M. May-Sept</td>
<td>100</td>
<td>17324</td>
<td>11442</td>
<td>3909</td>
<td>865</td>
<td>53</td>
<td>-</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14.2)</td>
<td>(93.8)</td>
<td>(32.1)</td>
<td>(7.1)</td>
<td>(0.4)</td>
<td></td>
<td>(0.09)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>N-E M. Oct-Dec</td>
<td>94.31</td>
<td>7209</td>
<td>1290</td>
<td>8353</td>
<td>223</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>(0.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(65.5)</td>
<td>(11.7)</td>
<td>(75.9)</td>
<td>(2.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole year</td>
<td>95.88</td>
<td>35644</td>
<td>17897</td>
<td>15496</td>
<td>1439</td>
<td>53</td>
<td>11</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>of study</td>
<td></td>
<td>(76.5)</td>
<td>(38.4)</td>
<td>(33.2)</td>
<td>(3.1)</td>
<td>(0.1)</td>
<td>(0.02)</td>
<td>(0.03)</td>
<td>(0.03)</td>
</tr>
</tbody>
</table>

* Figures in parenthesis indicate intensity of infection

(Db: Dinurus barbatus, Dbr: D. breviductus, Dc: D. coryphaenae, Dh: D. hippuri,
Fig. 6

Graphical representation of *Dirinus* collected in 2002.

S-W: southwest.]
Table 5

Season wise data on *Dinurus* from *C. hippurus* in 2003

<table>
<thead>
<tr>
<th>Season</th>
<th>Incidence</th>
<th>Different species and their number</th>
<th>Dh</th>
<th>Dl</th>
<th>Dt</th>
<th>Db</th>
<th>Ds</th>
<th>Di</th>
<th>Dc</th>
<th>Dbr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>93.22</td>
<td></td>
<td>3290 (16.4)</td>
<td>2312 (11.5)</td>
<td>1892 (9.4)</td>
<td>214 (1.06)</td>
<td>-</td>
<td>11 (0.05)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jan-Feb</td>
<td></td>
<td></td>
<td>16.4</td>
<td>11.5</td>
<td>9.4</td>
<td>1.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Summer</td>
<td>95.83</td>
<td></td>
<td>5345 (26.7)</td>
<td>6532 (32.7)</td>
<td>1390 (6.9)</td>
<td>591 (2.9)</td>
<td>-</td>
<td>2 (0.01)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mar-May</td>
<td></td>
<td></td>
<td>26.7</td>
<td>32.7</td>
<td>6.9</td>
<td>2.9</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S-W M.</td>
<td>100</td>
<td></td>
<td>18753 (91.5)</td>
<td>11900 (58)</td>
<td>9277 (45.3)</td>
<td>434 (2.12)</td>
<td>23 (0.1)</td>
<td>-</td>
<td>9 (0.04)</td>
<td>57 (0.03)</td>
</tr>
<tr>
<td>May-Sept</td>
<td></td>
<td></td>
<td>91.5</td>
<td>58</td>
<td>45.3</td>
<td>2.12</td>
<td>0.1</td>
<td>-</td>
<td>0.04</td>
<td>-</td>
</tr>
<tr>
<td>N-E M.</td>
<td>94.31</td>
<td></td>
<td>1123 (5.6)</td>
<td>3234 (16)</td>
<td>3340 (16.5)</td>
<td>171 (0.8)</td>
<td>-</td>
<td>-</td>
<td>34 (0.2)</td>
<td>9 (0.04)</td>
</tr>
<tr>
<td>Oct-Dec</td>
<td></td>
<td></td>
<td>5.6</td>
<td>16</td>
<td>16.5</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>Whole year of study</td>
<td>96.65</td>
<td></td>
<td>28511 (35.3)</td>
<td>23978 (29.7)</td>
<td>15899 (19.7)</td>
<td>1410 (1.7)</td>
<td>23 (0.02)</td>
<td>13 (0.02)</td>
<td>43 (0.05)</td>
<td>66 (0.08)</td>
</tr>
</tbody>
</table>

* Figures in parenthesis indicate intensity of infection


Northeast monsoon, S-W M: Southwest monsoon]*
Fig. 7

Graphical representation of *Dinurus* collected in 2003

DISCUSSION

Studies on the survey and seasonal occurrence of *Dinurus* are wanting and hence there is little baseline information for comparison with the present investigation. Fish and its parasites are such an association of organisms, in which the parasite (especially the endo-parasite) appears as the part of the internal medium of its host. It is known that many forms of parasites can be used as the indicators of the special features of biology and ecology of their masters, including of trophic connections, behaviour, depth of the habitat, migration, origin and zoogeography (Gayevskaya, 2000). Stomach of dolphin fishes is known to harbour a rich and diverse assemblage of hemiurid species. In the present study, out of 2,202 dolphin fishes collected from southwest coast of India and examined, only 68 were found uninfected. The results of the present study show that, the southwest coasts of Indian Sea harbour a diverse fauna of *Dinurus*. Of the 8 species of *Dinurus*, *D. ivanosi* is new to science, named and reported by Rekha & John (2004). *D. hippuri* was already recorded from Indian dolphin fishes (Nadakal et al., 1991). The remaining six species, though known from other geographical regions, are reported for the first time from dolphin fishes of India. *D. euthynni* reported by Yamaguti (1934) is the one reported species which could not be recovered in the present study. This is also the first report of a new host of *D. scombri* as this species has not been reported from *C. hippurus*.

In this study, two, three or even four species of *Dinurus* were recovered from the same host, and hosts with single species were rare. Fischthal & Thomas (1971) reported *D. tornatus, D. breviductus* and *D. barbatus* from the same host,
C. hippurus and Manter (1947) recorded four species including D. longisinus from the same. Parasites can be valuable as means of tracing food-web relationships and food-web structure, as well as studying the diversity of their habitats (Marcogliese & Cone, 1997; Marcogliese, 2001). Many biologists overlook the potential for using the presence or absence of helminth parasites as indicators of trophic interactions and other aspects of their host’s biology. Many helminth parasites have complex life cycles involving intermediate hosts, and typically exploit food web relationships, particularly predator-prey interactions for transmission. Thus, many parasites can be used as "biological tags" and provide valuable information on the feeding habits and patterns of habitat use of their vertebrate hosts. Parasites can also be excellent indicators of environmental contaminants and stress, especially in aquatic ecosystems (MacKenzie et al., 1995). This can, especially be important for assessing the host-parasite systems, since the hosts themselves can be valuable bioindicators (Bonin et al., 1995). In all these cases it is necessary to understand several aspects of host ecology and parasite phylogeny, host specificity and life cycle dynamics.

According to Kraul (1999) epipelagic and highly migratory coryphaenids are distributed in tropical and subtropical oceans. Different groups of the hosts may migrate towards the Indian peninsula in different seasons. Seasonal fluctuations in helminth metapopulations are well documented by Marcogliese et al. (1982) and it is generally assumed that dynamic changes occur even within infrapopulations. Seasonal abundance of dolphin fish, C. hippurus might be a function of cohort (group) survival. The strongest cohorts of them were reported to spawn in July and
stay in the fishing zone of Pacific Ocean for at least 5 months (Kraul, 1999). The abundance pattern also fits the pattern of change in seasonal surface temperatures, and it is possible that the specimens of *C. hippurus* migrate north and south to stay in the sea surface with a temperature of about 23°C. This temperature may also be conducive to the abundance of the pelagic organisms which act as intermediate hosts of the *Dinurus* species.

Seasonal fluctuations of trematode infection have been well documented in temperate regions. The results of these studies suggest that seasonal differences in the incidence of infection and mean intensity of parasites in fishes are attributable to fluctuations in water temperature, host behaviour, parasite population density, changes in host population structure and host trophic variations (Muzzall *et al.*, 1992; Campos & Carbonell, 1994). According to Bauer & Karimov (1990) temperature is not at all a major factor influencing the nature of parasitisation of fishes. Studies by Madhavi (1979) and Beevi (1993) have revealed only indefinite and inconsistent patterns of seasonal differences in parasitic infections of tropical fishes. However, they recorded slightly higher incidence and mean intensity of infection during the rainy months. In the present study *D. ivanosi* infections were registered inconsistent patterns in the yearly cycle.

Chubb (1979) emphasized the importance of the temperature as a factor affecting the population biology of trematode parasites infecting the ectotherms in aquatic ecosystem. The incidence of *D. hippoc, D. longisinus* and *D. barbatus* infection was high in the summer and southwest monsoon, while that of *D. tornatus* high during monsoons. There is a general tendency for a decrease in the incidence
and intensity of infection during summer season. Campbell (1974) studied seasonal changes of *Ichtyocotylurus erraticus*, a trematode parasite of *Salmo trutta* and observed lowest incidence in summer and highest in winter.

*C. hippurus* has been reported in the central-east Atlantic, including the Canary Islands (Pujolar & Pla, 2002). A connection between Atlantic and Mediterranean *C. hippurus* has been known since ancient times and has recently been demonstrated with parasite studies (Carbonell *et al*., 1999). They are reported to spawn in the open sea (when the water temperature rises) in July (Palko *et al*., 1982). Monsoon water of Kerala coast is reported to have a rich fauna of crustaceans and cephalopods. Hence a variety of fishes migrate towards southern peninsula for spawning and feeding (Farlex, 2004). Fishes usually prefer to feed in the most energetically rewarding habitat available (Werner & Mittelbach, 1981), where benefits include calories and nutrients ingested (Helfman *et al*., 1997).

Abundance of intermediate host in the environment helps the spread of infection. Higher incidence and intensity of infection observed during the monsoon might be due to high rate of fresh infection following periods of fairly good rainfall received in the southwest and northeast monsoons. In Kerala, the winter season includes the months of January and February. This is followed by summer months March, April and May. The rest of the year is the rainy season which is divided into the southwest monsoon (June, July, August and September) and the northeast monsoon (October, November and December). Southwest and northeast monsoons are characterized by moderately heavy rain fall. George & Nadakal (1980) and Samuel (1993) also observed correlation between peak infection of fish parasites
and rainy seasons which favour the breeding and growth of the definitive as well as the intermediate hosts. Walkey (1967) reported irregularities in the seasonal occurrence of intermediate host which lead to fluctuations in *Neoechinorhynchus rutili* (Acanthocephala) population. In the present study juveniles of all the *Dinurus* were observed during May to August, indicating fresh infection.

Feeding habit of the host also varies according to seasons. Merrit & Haedrich (1997) reported the feeding habit of dolphin fishes. These fishes feed heavily on fishes, pelagic cephalopods, and crustaceans. The present study shows a difference in the stomach contents of the hosts in various seasons. Stomachs collected during summer were found to have small and medium sized fishes. Crabs and sepia were abundant during monsoon. Host body size is not a good predictor of the abundance and richness of parasite infra-communities (Muñoz et al., 2001). However, the present study revealed that large hosts were rich in parasite fauna compared to the small. Larger fishes generally tend to have higher rate of infection (Muzzall et al., 1992; Holmes & Bartoli, 1993). Rabidenu & Self (1953) and Fox (1962) reported that the larger the host, the higher the number and the greatest the variety of parasites invading it. Buchmann (1989) observed positive correlation between host size and the number of parasites. Higher infection rate with increase in size of host fish has also been recorded for digenean trematodes (Kennedy & Burrough, 1977; Kalantan et al., 1987). According to them, large fishes would provide large space for parasitic invasion. Increase in infection levels with increase in fish size may be due to changes in the host diet (Holmes, 1990). In addition, large fishes are presumed to consume more and varied food, including the
intermediate hosts of the parasites, thereby increasing the chances of getting infected (Holmes & Bartoli, 1993). However, Holmes (1990) found no difference or change in the parasite fauna with the size or growth of the host. A progressive decrease in the parasite fauna with increase in host size observed in some cases may be because of the unbalanced recruitment and death of parasites (Muzzall et al., 1992), inability of the host to support large parasite burdens (Pennycuick, 1971), or the inefficient immune system of the older fishes (Holmes, 1990).

In the present study all the *Dinurus* reported were recovered from stomach of the host. However, Dyer et al. (1998) reported the occurrence of *D. barbatus* from the intestine of *C. hippurus* in Puerto Rica. The intestine may not be the normal microhabitat of this species but this rare observation may be the aftermath of lapse of time between the collection of the host fish and recovery of the worms. Dyer et al. (1998) agreed that their studies were based on fishes collected from a distant market. This indicates the possibility of the migration of *Dinurus* from stomach to intestine. In the present study, the parasites were found dead after six hours of the death of host.

*D. barbatus, D. breviductus, D. coryphaenae, D. scombri* and *D. ivanosi* do not seem to be the frequent parasites of *C. hippurus* as evident from the incidence and intensity of infection. The exact reasons for this lean nature of fauna of this coast remain elusive. Probably, the non-availability of the secondary host species throughout the year along this coast may be one of the reasons.
A note on crowding effect on *D. hippuri*

In the present study crowding effect, a phenomenon which may be related to nutrition in most of the helminths, was observed in *D. hippuri* infection. The worms from low and high density infections (5-10 worms/fish and 50-1000 worms/fish) were compared. Worms of high density infections were smaller. The egg production per worm was less than the parasites in low density infections.

Crowding effect has been described in almost all trematodes, but has been most extensively studied in the rat tape worm, *Hymenolepis diminuta* (Roberts & Mong, 1973). There are relatively few experimental studies on the effects of intraspecific crowding of digeneans (Fried & Nelson, 1978; Mohandas & Nadakal, 1978; Nollen, 1983; Fried & Freeborne, 1984; Franco et al., 1988). Several possibilities have been suggested to account for the crowding effect. It could be due to local immunity, to competition for nutrients or carbon dioxide or oxygen, to actual physical crowding or to the mutual inhibition of worm growth by secretary or excretory products. Presence of other species of helminths in the same host may also cause crowding effect because all these worms have an absolute requirement for carbohydrates. Mohandas & Nadakal (1978) found that crowding reduced the length of *Echinostoma malayanum* in rats. Similarly Fried & Nelson (1978) found that chickens infected with single adults of *Zygocotyle lunata* grown in the caeca were twice as long as worms from infections of 30 or more. It has been suggested that chemical “factors” released by worms associated with crowding may be important influences on worm growth (Fischthal et al., 1982; Roberts & Insler, 1982).
SUMMARY

Eight species of *Dinurus* recovered from *Coryphaena hippurus* during the study, were *D. hippuri*, *D. longisinus*, *D. tornatus*, *D. barbatus*, *D. scombri*, *D. breviductus*, *D. coryphaenae* and *D. ivanosi*. Although many hosts were infected with more than one species of *Dinurus*, those having a combination of *D. tornatus*, *D. hippuri* and *D. longisinus* were common. Hosts with single species were rare. The intensity of infection was high during monsoon season. In the season wise analysis it was found that the incidence of *D. tornatus* infection was generally high in the rainy seasons, while that of *D. hippuri*, *D. longisinus* and *D. barbatus* high in the summer and southwest monsoon. *D. breviductus*, *D. scombri*, *D. coryphaenae* and *D. ivanosi* had no definite seasonal pattern. Though *D. barbatus* was recovered in all seasons, the intensity of infection was low compared to *D. hippuri*, *D. longisinus* and *D. tornatus*. *D. ivanosi* was mostly encountered in winter and summer, while *D. scombri* only in southwest monsoon. *D. hippuri* and *D. ivanosi* were reported only from India. The remaining six species, although known from other geographical regions, are reported for the first time from India. *C. hippurus* is a new host of *D. scombri*. 
Chapter 2

DESCRIPTION OF DINURUS

INTRODUCTION

Earlier descriptions on the different species of *Dinurus* were vague and brief, primarily intended to report them from different hosts and geographical locations and hence room for controversies regarding their structural details and species status. Some authors including Dawes (1947), Ward (1954) and Zhukov (1977) have considered all the *Dinurus* species from dolphin fish synonymous. Bray (1990) considered only the sucker width ratio to separate *D. longisinus* from *D. tornatus* as opposed to other distinguishing characters highlighted by Gibson (1976). Nadakal *et al.* (1991) reported *D. hippuri* from *C. hippurus*. Bray *et al.* (1993) challenged the species status of *D. hippuri* and considered it as synonym of *D. longisinus* on account of the similarity of seminal vesicle and the egg-size. In the ‘European Register of Marine Species’ Bray & Gibson (1999) included only three species *D. breviductus*, *D. scombri* and *D. tornatus*. Because of the controversies, it was thought desirable to study all the *Dinurus* species and enlist their distinguishing features in the present work.

MATERIALS AND METHODS

The worms from fresh specimens of *C. hippurus* were transferred to 0.6% saline. Different *Dinurus* species were sorted out (mature and immature worms of
each separately) with the help of hand lens and dissection microscope. To study live samples, the worms were kept at 15°C in saline solution with 0.1% glucose. They were also fixed in 10% neutral formalin as well as 70% alcohol, aqueous Bouin’s and alcoholic Bouin’s for later use. Worms for whole mounts were pressed using slight cover glass pressure to prevent curling, fixed in 10% buffered formalin overnight, stained with borax carmine, dehydrated in graded series of alcohol, cleared in methyl benzoate and mounted in DPX. Figures were drawn with the aid of camera lucida and the measurements (average of 30 specimens, except for *D. ivanosi*, which include 24 specimens) given are in micrometers (µm), unless otherwise mentioned. The list of fixatives and stain used and their recipe are given in Table 6. References for species identification are listed in Table 7.

The *Dinurus* species collected have been deposited in Helminthology collections in the Department of Zoology, Mar Ivanios College, Trivandrum.

OBSERVATIONS

The eight species of *Dinurus* recovered from the stomach of *C. hippurus* were studied for their taxonomic identity. The diagnostic features of *Dinurus* are the division of the body into an anterior prosoma and a posterior ecsoma, presence of prosomal plications, smaller size of oral sucker, post-acetabular tri or quadripartite seminal vesicle, two pre-ovarian testes and seven long, tubular vitellaria. The distinguishing features of these worms with special reference to those noted for the first time are shown in Table 8.
**Dinurus hippuri** (Figs. 8A, B)

A detailed description of the morphology of *D. hippuri* is presented in Chapter 3 of this thesis. However, in the present chapter this species is compared with the other species of *Dinurus* recovered from Kerala.

**Dinurus longisinus** (Figs. 9A, B)

Body appeared slim with rounded anterior and almost tapering posterior extremity. Prosoma 6.84 mm, shorter than the ecsoma 9.19 mm. Prosomal tegument covered with a carpet of plications from behind the oral sucker. Papillae absent. Oral sucker 345 x 345, terminal and cup-shaped, pre-oral lobe absent. Ventral sucker 784 x 798, placed 824 behind the oral sucker. Cavity of ventral sucker directed forward. Size of the ventral sucker always more than twice the size of oral sucker. Sucker ratio 1:2.18 - 2.29. Pharynx sub-globular, measured 199 x 186. Oesophagus distinct, long and highly contractile. Intestinal caeca simple, paired, terminated just above the excretory bladder. Excretory system consisted of four collecting vessels, excretory bladder and excretory pore situated at the end of ecsoma.

Testis paired, oval and more or less tandem. Posterior testis placed 26 - 53 behind the anterior testis. Anterior testis measured 571 x 465 and the posterior 545 x 492. Seminal vesicle oblong and trilobed, measured 571 x 252, placed 2,194 behind the ventral sucker. The middle lobe, 226 x 252, was larger than the distal, 172 x 252, and proximal, 172 x 252. Pars prostatica 3,152 x 53, wavy, extended nearly half of the ventral sucker length. Prostatic gland cells were comparatively

38
smaller, arranged throughout the pars prostatica leaving the two ends free of them. Sinus sac 691 x 10, long and distinct. Ovary single, slightly parabolic, measured 359 x 399. Ootype, 319 x 332, mostly overlapped with posterior end of ovary. Vitellaria long, slender, coiled tubes; the longest being 4,788 x 53-66, three of them directed forward and the remaining backward to two-third of the ecsoma. Forwardly directed vitellaria were highly coiled hence vitelline field minimum. Uterus descended half to the ecsoma. Eggs 17 x 11, thin walled and embryonated.

Dinurus tornatus (Figs.10, 11)

Body stout, broadest at the prosoma - ecsoma junction, measured 1.20 mm, where the overhanging part of the prosoma projected prominently. The ecsoma, 8.73 - 9 mm was about twice as long as prosoma, 5 - 5.16 mm and constricted a number of times at irregular intervals. Plications were prominent and arranged uniformly throughout the prosoma. Oral sucker 532 x 452, elliptical and sub-terminal. Distinct pre-oral lobe present (Fig. 11A). Ventral sucker, 731 x 798, placed 731 from oral sucker. Sucker ratio 1:1.36 - 1.55. Pharynx 186 x 226, continued to short oesophagus. Intestinal caeca terminated blindly just above the excretory sac. Single, large, median excretory duct extended from prosoma to ecsoma.

Testis paired, sub-globular, oblique and tandem. Anterior testis 372 x 412 and posterior, 425 x 385. Seminal vesicle, 438 x 218, placed 665 from the ventral sucker. Thick wall of the seminal vesicle often made the lobes morphologically indistinct but with three lobes, middle lobe being small (Fig. 11C). Seminal vesicle
continued to pars prostatica, with two and a half turns, extended nearly half of the ventral sucker length (Fig. 11D). Large prostatica cells were closely arranged throughout the length of pars prostatica. Pars prostatica entered into the large and vesicular sinus sac which measured 798 x 226 (Fig. 11B). Sinus sac carried a common hermaphroditic duct within, which received both male and female ducts. The paired sinus organs were found attached to the tip of the hermaphroditic duct (Fig. 11D). Sinus organs 119 – 218 x 19, short. A muscular sphincter was noted in the portion of sinus sac where sinus organs meet. The male duct opened into a large and distinct atrium. A muscular papillated cirrus present at the terminal portion of the male reproductive duct.

Ovary single, spherical, 292 x 332, placed 332 behind the posterior testis and 465 anterior to the prosoma-ecsoma junction. Ootype 252 – 266 x 279, sub-globular with conspicuous Mehlis’ gland cells. Vitelline field conspicuous. Three of the seven vitellaria directed anterior to the ovary. Four vitellaria directed backward on either side of the ootype, extended 1,150 - 2128 to ecsoma. Each vitellarium measured 3,325 x 66 - 79. Uterus well developed, descending limb extended 5,253 - 5,320 to ecsoma. Eggs numerous and thick shelled, measured 9 x 13.
**Dinurus barbatus** (Figs. 12, 13)

Prosoma 4.16 mm, longer than the ecosoma 3.86 mm. Body stout with a straight fore body. Plications arranged uniformly on the prosoma. A tuft of tubular integumentary outgrowths present just behind the oral sucker, ventrally. The number ranged from 18 - 24, of which 6 - 8 comparatively longer, 239 x 13. Suckers almost circular. Oral sucker 385 x 372, ventral sucker 877 x 811, 904 from oral sucker. The sucker ratio was 1:2.17 - 2.22. Distinct pre-oral lobe present. Papillae scattered throughout the sucker regions. Pharynx measured 146 x 186. Indistinct oesophagus bifurcated into intestinal caeca from behind the pharynx. Excretory opening at the end of ecosoma.

Testis paired, globular, oblique and close together, mostly overlapping (Fig. 13B). Anterior testis, 425 x 452, 571 behind the ventral sucker. Posterior testis, 505 x 505. Seminal vesicle 438 x 172, thin walled, trilobed, nearly 66 - 133 from the ventral sucker. The three lobes were equal in size and shape, measured 146 x 172 (Fig. 13C). Pars prostatica long and less coiled. Prostatic gland cells small and arranged throughout the pars prostatica except the two ends. Pars prostatica passed forward through the side of the ventral sucker, joined the sinus sac. Sinus sac very short, led to a short genital atrium (Fig. 13A), usually masked by the integumentary outgrowths.

Ovary single, oval, measured 199 x 279. Ootype globular 218 x 199. Vitellaria, seven, two directed forward, the rest backward. Mostly vitellaria found coiled together and confined to the prosoma with less vitelline field. Uterus very
short, descended down 1,901 - 2088 to ecsoma, then turned forward to enter the sinus sac (Fig. 12B). Eggs measured 15 - 19 x 19 - 22.

**Dinurus scombri** (Figs. 14, 15)

Body straight, slightly broad at the level of ventral sucker (Fig. 14 B). The prosoma 6 mm and ecsoma 5 mm in length. Prosomal plications were distributed from below the level of ventral sucker. Suckers were almost of same size (Fig. 15 A, B). Oral sucker 452 x 478, sub-terminal with pre-oral lobe of 13 long. Ventral sucker 505 x 492, 931 from oral sucker. Sucker ratio was 1:1 - 1. 07. Bulbous pharynx, 199 x 172. Oesophagus indistinct, two intestinal caeca extended nearly to the posterior end of ecsoma.

Testis paired, globular and adjacent, 638 below the ventral sucker and posterior to the seminal vesicle. Anterior testis 399 x 372 was smaller than the posterior 492 x 425. Seminal vesicle 465 long, 133 from ventral sucker and with four unequal lobes (Figs. 15C, D). Distal lobe 119 x 133, first middle lobe smallest 53 x 146, second middle lobe 133 x 199, and proximal lobe 159 x 186. Pars prostatica with small prostatic gland cells, the two ends devoid of them, extended above the ventral sucker to enter the sinus sac. Sinus sac very short, 691 x 10, lead to genital pore at the lower margin of oral sucker, opened out through short genital atrium.
Ovary single, globular, 239 x 239, placed 266 behind the posterior testis and 1,542 above the prosoma-ec soma junction. Ootype 133 x 146, found attached to ovary. Vitellaria were seven scattered coiled tubes confined to prosoma. The uterine coils extended 824 to ecsoma, the major folds of the ascending limb confined to the prosoma (Fig. 14.A). The ascending limb turned above the ventral sucker level to enter the sinus sac. The eggs thin shelled, oval and measured 13 x 6.

*Dinurus coryphaenae* (Figs.16, 17)

Body 9 mm in length, prosoma being 3.6 mm. The tegumental placations very prominent and present all over the prosoma. Numerous papillae scattered throughout the fore body (Fig. 17B). Oral sucker, 665 x 745. Pre-oral lobe present, 8 in length. Ventral sucker 1103 x 1077, 1,289 from oral sucker. Acetabular cavity directed forwards (Fig. 17A). Sucker ratio was 1:1.51. The pharynx highly muscular and longer than broader, 218 x 172 (Fig. 17B). The oesophagus short, so that the intestinal bifurcation took place immediately behind the pharynx. The intestinal caeca extended to the posterior extremity of ecsoma.

Testis paired, closely arranged one behind the other. The anterior testis 691 x 704 while the posterior 704 x 731. The seminal vesicle 718 from ventral sucker, thin walled with three equal lobes. Pars prostatica straight running forward through the side of the ventral sucker. Small prostate gland cells carpeted the pars prostatica leaving the two ends aglandular. Sinus sac very short oblong 691 x 13,
highly muscular and located near the oral sucker (Fig. 17C). Sinus organ indistinct, the genital atrium long.

Ovary single, 365 x 465 and globular. Ootype globular, 226 x 359. Vitellaria seven, two directed forward and shorter than the rest (Fig. 17A). Backwardly directed vitellaria extended 729 to ecsoma. Uterus extensive, occupying almost all available space in the body. Uterine limb descended 2/3 of the ecsoma. Egg 19 x 11, thin walled.

**Dinurus breviductus** (Fig. 18)

Body slender, 12.33 mm in length. Prosoma 8.32 mm, longer than the ecsoma. Anterior limit of prosomal placations was the lower margin of oral sucker. Oral sucker terminal, 492 x 425 and the pre-oral lobe absent. Ventral sucker 1,010 x 958, 1,410 away from the oral sucker. The sucker ratio was about 1:2. Pharynx globular, 133 x 146. Oesophagus indistinct and the intestinal caeca extended to posterior region of ecsoma.

Testes globular, each with a cone shaped portion at the junction between the testis and vas deferens (Fig. 18B). Anterior and posterior testes were of equal size and shape, 505x492. Vasa deferentia were distinct long tubes joined at the basal lobe of the seminal vesicle. Seminal vesicle trilobed, 1,064 x 345. The large anterior and posterior lobes were equal in size 412 x 345, while the middle lobe small and measured 239 x 345. Pars prostatica more or less straight running forward to the ventral sucker region. The prostate gland cells were small, but well
developed and closely packed. The two ends of pars prostatica were aglandular. Sinus sac 824 x 172, and positioned anterior to the ventral sucker. Sinus organ long and distinct, measured 784 x 79. Genital atrium present which opened below the oral sucker.

Ovary single, globular, 226 x 252, situated almost at the posterior limit of prosoma. Ootype small and indistinct. Vitellaria less coiled, two directed forward, the remaining backward, extending to half of ecsoma. Uterus well developed, highly coiled, running down to the ecsoma (Fig. 18A). Eggs numerous, measured 25 x 20.

*Dinurus ivenosi* (Figs. 19, 20)

Body slightly curved at the anterior end and tapering at both ends. Prosoma and ecsoma were well demarcated. Integumentary plications of the prosoma were large and uniformly arranged. Anterior limit of the placation was anterior margin of oral sucker (Fig. 20B). Prosoma 3.04 – 3.47 mm long, roughly conical and shorter than ecsoma. Ecsoma 5.08 – 5.187 mm long, smooth and almost straight. Oral sucker 159 – 252 x 226 – 332 was terminal and pre-oral lobe absent. Ventral sucker 412 – 339 x 345 – 399; 399 – 465 from oral sucker. Cavity of the ventral sucker directed forward. Sucker ratio 1:1.54. Pharynx 665 – 691 x 199–239 and elongated (Fig. 20A), emerging into oesophagus which measured 106 – 133 x 66 – 79 and bifurcated into straight, long, tubular, downwardly directed, blind intestinal caeca ending in ecsoma.
Testis paired, parabolic, oblique, contiguous and pre-ovarian. Anterior testis, $252 - 385 \times 438 - 532$, larger than the posterior, $239 - 399 \times 345 - 359$ (Fig. 20C). Anterior testis placed $385 - 651$ behind the ventral sucker. Vas deferens inconspicuous. Seminal vesicle well developed, oblong $465 - 532 \times 199 - 226$; $332 - 425$ from ventral sucker. Seminal vesicle thick walled and trilobed; placed adjacent to anterior testis. Distal lobe $119 \times 199$ was smaller than the middle $146 \times 226$ and the proximal $199 \times 199$ (Fig. 20D). Pars prostatica, $585 - 665 \times 133 - 172$, extended forward to ventral sucker region. Small prostatic gland cells carpeted around the duct, leaving proximal and distal ends, non-glandular. Sinus sac very short and indistinct, placed far above the level of ventral sucker.

Ovary parabolic, $172 - 159 \times 292 - 266$, post testicular by $218 - 305$. Ootype attached to ovary. Vitellaria represented thin and short distinct ducts, with less vitelline field. Vitellaria seven, two directed anteriorly, rest posteriorly $704 - 1197$ to ecsoma (Fig. 20E). Uterus originating from behind the ootype, and less coiled, descending $3.29 - 3.4$ mm to ecsoma; then turning forward to enter the sinus sac. Eggs $15 \times 9$, thin walled and oval. Excretory ducts, four, continued backwards through lateral margin of the body to the ecsoma.
<table>
<thead>
<tr>
<th>Saline/Fixative/stain</th>
<th>Recipe</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Dissolve 0.1g glucose in 0.6% NaCl. To 100 ml of this, dissolve 6.10 g Na₂HPO₄ and 0.15 g</td>
<td>(Horobin, 1988)</td>
</tr>
<tr>
<td>10 % buffered formalin</td>
<td>NaH₂PO₄ in 800ml 37.5% formaldehyde + 7200 ml water</td>
<td>(Ash &amp; Orihel, 1991)</td>
</tr>
<tr>
<td>Bouins’ fluid</td>
<td>Mix 75 ml sat.aq. picric acid in 25 ml formalin. Just before use add 5ml acetic acid</td>
<td>(Pearse, 1968)</td>
</tr>
<tr>
<td>Alcoholic Bouins’</td>
<td>Dissolve 1g picric acid in 60ml formalin+ 15 ml acetic acid +150 ml 80 % ethanol</td>
<td>(Pearse, 1968)</td>
</tr>
<tr>
<td>70 % ethanol</td>
<td>740 ml 95 % ethanol + 260 ml water</td>
<td>(Ash &amp; Orihel, 1991)</td>
</tr>
<tr>
<td>Borax carmine</td>
<td>Mix 3.5 g borax carmine in 100 ml 70 % ethanol, stir warm and filter</td>
<td>(Pearse, 1968)</td>
</tr>
<tr>
<td>Toluidine blue</td>
<td>Dissolve 1g toluidine blue in 100 ml distilled water and filter</td>
<td>(Horobin, 1988)</td>
</tr>
</tbody>
</table>
### Table 7
Useful references for identification of *Dinurus* species

<table>
<thead>
<tr>
<th><em>Dinurus</em> species</th>
<th>Reference</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td><em>D. tornatus</em></td>
<td>Looss, 1908</td>
<td>Useful</td>
</tr>
<tr>
<td></td>
<td>Yamaguti, 1971</td>
<td>Only drawing</td>
</tr>
<tr>
<td><em>D. barbatus</em></td>
<td>Gibson, 1976</td>
<td>Excellent</td>
</tr>
<tr>
<td><em>D. breviductus</em></td>
<td>Gibson, 1976</td>
<td>Good for identification</td>
</tr>
<tr>
<td><em>D. longisinus</em></td>
<td>Bray, 1990 &amp; 1993</td>
<td>Useful</td>
</tr>
<tr>
<td></td>
<td>Rekha &amp; John, 2002a</td>
<td>Good for identification</td>
</tr>
<tr>
<td><em>D. coryphaenae</em></td>
<td>Yamaguti, 1934</td>
<td>Excellent</td>
</tr>
<tr>
<td><em>D. scombri</em></td>
<td>Yamaguti, 1934</td>
<td>Excellent</td>
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<td></td>
<td>Léon-Régagnon <em>et al.</em>, 1997</td>
<td>Useful</td>
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<td><em>D. hippuri</em></td>
<td>Nadakal <em>et al.</em>, 1991</td>
<td>Poor drawings</td>
</tr>
<tr>
<td></td>
<td>Rekha &amp; John, 2002a</td>
<td>Good for identification</td>
</tr>
<tr>
<td><em>D. ivanosi</em></td>
<td>Rekha &amp; John, 2004</td>
<td>Excellent</td>
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## Table 8
The distinguishing features of *Dinurus* species

<table>
<thead>
<tr>
<th>No.</th>
<th>Characteristics</th>
<th><em>Dinurus hippuri</em></th>
<th><em>Dinurus longisinus</em></th>
<th><em>Dinurus tornatus</em></th>
<th><em>Dinurus barbatus</em></th>
<th><em>Dinurus scorbri</em></th>
<th><em>Dinurus coryphaenae</em></th>
<th><em>Dinurus breviductus</em></th>
<th><em>Dinurus ivanosi</em></th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total length (in mm)</td>
<td>7</td>
<td>16</td>
<td>14</td>
<td>8</td>
<td>11</td>
<td>9</td>
<td>12.3</td>
<td>8.5</td>
</tr>
<tr>
<td>2.</td>
<td>Relative length of prosoma-ecssoma</td>
<td>Prosome &amp; ecssoma more or less similar in length</td>
<td>Prosome shorter than ecssoma</td>
<td>Prosome shorter than ecssoma</td>
<td>Prosome longer than ecssoma*</td>
<td>Prosome longer than ecssoma*</td>
<td>Prosome shorter than ecssoma</td>
<td>Prosome shorter than ecssoma</td>
<td>Prosome shorter than ecssoma</td>
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<td>3.</td>
<td>Pre oral lobe</td>
<td>Present*</td>
<td>Absent*</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>4.</td>
<td>Anterior limit of plications</td>
<td>Posterior to oral sucker*</td>
<td>Just posterior to oral sucker</td>
<td>Anterior margin of oral sucker*</td>
<td>Anterior margin of oral sucker*</td>
<td>Anterior margin of oral sucker*</td>
<td>Anterior margin of oral sucker*</td>
<td>Anterior margin of oral sucker*</td>
<td>Anterior margin of oral sucker*</td>
</tr>
<tr>
<td>5.</td>
<td>Sucker ratio (based on mean diameter)</td>
<td>1:1.68-1.80</td>
<td>1.28-2.29</td>
<td>1:1.36-1.55</td>
<td>1:2.17-2.22</td>
<td>1:1-1.07</td>
<td>1:1.51</td>
<td>1:2</td>
<td>1:1.54</td>
</tr>
<tr>
<td>6.</td>
<td>Nature of seminal vesicles*</td>
<td>Thin walled, trilobed with small middle lobe</td>
<td>Thin walled, trilobed with small middle lobe</td>
<td>Thin walled, three lobes of equal size</td>
<td>Thin walled, four lobes of unequal size</td>
<td>Thin walled, trilobed with small middle lobe</td>
<td>Thin walled, trilobed with small middle lobe</td>
<td>Thick walled, trilobed with small distal lobe</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Nature of sinus organ</td>
<td>Long</td>
<td>Long</td>
<td>Short</td>
<td>Short</td>
<td>Short</td>
<td>Short</td>
<td>Long</td>
<td>Short</td>
</tr>
<tr>
<td>8.</td>
<td>Posterior limit of sinus sac</td>
<td>Midlevel of ventral sucker*</td>
<td>Mid level of ventral sucker</td>
<td>Forward to the ventral sucker*</td>
<td>Forward to the ventral sucker*</td>
<td>Forward to the ventral sucker*</td>
<td>Forward to the ventral sucker*</td>
<td>Forward to the ventral sucker*</td>
<td>Forward to the ventral sucker*</td>
</tr>
<tr>
<td>9.</td>
<td>Nature of Testes*</td>
<td>Globular and contiguous</td>
<td>Sub-global and oblique</td>
<td>Globular and overlapping</td>
<td>Globular and contiguous</td>
<td>Globular and contiguous</td>
<td>Globular and contiguous</td>
<td>Global, one behind the other</td>
<td>Globular contiguous</td>
</tr>
<tr>
<td>10.</td>
<td>Nature of vitellaria*</td>
<td>Two directed anteriorly</td>
<td>Three directed anteriorly</td>
<td>Three directed anteriorly</td>
<td>Clumped, and limited in prosoma</td>
<td>Clumped, and limited in prosoma</td>
<td>Two directed anteriorly</td>
<td>Two directed anteriorly</td>
<td>Two directed anteriorly</td>
</tr>
<tr>
<td>11.</td>
<td>Egg size (in µm)</td>
<td>16x11</td>
<td>17x11</td>
<td>19x13</td>
<td>17x20</td>
<td>13x6</td>
<td>19x11</td>
<td>25x20</td>
<td>15x9</td>
</tr>
<tr>
<td>12.</td>
<td>Special features</td>
<td>Acetabular papillae and prominence*</td>
<td>Anteriorly directed, cup-shaped suckers*</td>
<td>Single, large, median excretory duct*</td>
<td>Large integumentary outgrowths below the oral sucker</td>
<td>Short descending limb of uterus*</td>
<td>Anteriorly directed, cup-shaped suckers*</td>
<td>Large seminal vesicle &amp; anterior elevation of testes</td>
<td>Elongated pharynx</td>
</tr>
</tbody>
</table>

* Features first reported by the present author
**Key to species identification**

1a Prosoma longer than ecsoma

1b Prosoma and ecsoma more or less similar in length

1c Prosoma shorter than ecsoma

2a Body thick and stout

2b Body slender and elongated

3 Long tubular projections just below the oral sucker

4a Oral and ventral suckers equal sized; seminal vesicle four lobed

4b Sucker ratio 1:2 or more

5 Six acetabular papillae; trilobed seminal vesicle with small middle lobe

6a Long sinus sac extend mid-level of ventral sucker; three ducts of vitellaria directed anteriorly

6b Short sinus sac extend above the level of ventral sucker; Two ducts of vitellaria directed anteriorly

7a Pre-oral lobe absent; long sinus organ; four slender, longitudinal excretory ducts; Sucker ratio 1:2 or more

7b Pre-oral lobe present; short sinus organ; single large, median excretory duct

8a Elongated pharynx; testes parabolic; large anterior testes

8b Short pharynx; globular testis; small anterior testis

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Fig. 8 A  D. hippuri: Camera lucida drawing
B Micrograph of stained (Borax carmine) whole mount
AG acetabular prominence, AP acetabular papilla, E ecsoma, IC intestinal caecum, OO ootype, 
OS oral sucker, OV ovary, P prosoma, PH pharynx, PP pars prostatica, SO sinus organ, SS sinus 
sac, SV seminal vesicle, UT uterus, VS ventral sucker, VT vitellarium
Fig. 9 A  *D. longisinus*: Camera lucida drawing

**B** Micrograph of stained (Borax carmine) whole mount

AT atrium, E ecosoma, IC intestinal caecum, OO ootype, OS oral sucker, OV ovary, P prosoma, PH pharynx, PP pars prostatica, SO sinus organ, SS sinus sac, SV seminal vesicle, UT uterus, VS ventral sucker, VT vitellarium
Fig. 10  *D. tornatus*: Camera lucida drawing
AT atrium, EC escoma, ED excretory duct, EV excretory sac, IC intestinal cæcum, OO ootype, OS oral sucker, OV ovary, P prosoma, PH pharynx, PP pars prostatica, SS sinus sac, SV seminal vesicle, UT uterus, VS ventral sucker, VT vitellariunm
Fig. 11  Micrographs of *D. tornatus*

A  Fore body under phase contrast microscope
B  Magnified view of sinus sac *(Toluidine blue)*
C  Prosoma under phase contrast microscope showing excretory duct & seminal vesicle
D  Prosoma (Toluidine blue stained) showing excretory duct & pars prostatica

AT atrium, CR cirrus, ED excretory duct, HD hermaphroditic duct, DL distal lobe, ML middle lobe, OS oral sucker, PH pharynx, PL proximal lobe, PP pars prostatica, SO sinus organ, SS sinus sac, SV seminal vesicle, VS ventral sucker
Fig. 12 A  *D. barbatus*: Camera lucida drawing

Fig. 12 B  *D. barbatus* (stained with borax carmine)

AT anterior testes, E ecsoma, EP excretory pore, IC intestinal caecum, IO integumentary outgrowths, P prosoma, PG prostate gland cells, PH pharynx, PT posterior testis, OO ootype, OS oral sucker, OV ovary, SS sinus sac, SV seminal vesicle, UT uterus, VS ventral sucker, VT vitellarium
Fig. 13 A  Micrograph showing fore body of *D. barbatus* (under phase contrast microscope)
B Micrograph of fore body (under phase contrast microscope)
C Camera lucida drawing of seminal vesicle

AT anterior testis, DL distal lobe, IO integumentary outgrowths, ML middle lobe, OS oral sucker, PH pharynx, PL proximal lobe, PP pars prostatica, PT posterior testis, SV seminal vesicle, VS ventral sucker, ♦♦ sinus sac
Fig. 14 A  *D. scombri*: Camera lucida drawing

B  Micrograph of prosoma (under phase contrast microscope)

AR atrium, AT anterior testes, E ecsoma, IC intestinal caecum, OO ootype, OS oral sucker, OV ovary, PT posterior testis, SS sinus sac, SV seminal vesicle, UT uterus, VT vitellarium
Fig. 15 A  Micrograph of fore body of *D. scombri* (under phase contrast microscope)

B  Micrograph of fore body of *D. scombri* (under phase contrast microscope)

C  Camera lucida drawing of quadripartite seminal vesicle

D  Seminal vesicle under phase contrast microscope

AT anterior testis, DL distal lobe, ML middle lobe, OS oral sucker, PH pharynx, PL proximal lobe

PP pars prostatica, PT posterior testis, SV seminal vesicle, VS ventral sucker, *UT urotrix*
Fig. 16 A  *D. coryphaena* : Camera lucida drawing

B  Micrograph of *D. coryphaena* showing prosoma (under phase contrast microscope)

- AR atrium, AT anterior testis, E ecsoma, IC intestinal caecum, PH pharynx, PP pars prostatica, PT posterior testis, OO ootype, OS oral sucker, OV ovary, SS sinus sac, SV seminal vesicle, UT uterus, VS ventral sucker, VT vitellarium
Fig. 17 A  Micrograph of *D. coryphaena* showing fore body (stained with borax carmine)
B  Anterior end of *D. coryphaena* under phase contrast microscope
C  Anterior end showing sinus sac (phase contrast micrograph)

AT atrium, PH pharynx, PP pars prostatica, OS oral sucker, SS sinus sac, SV seminal vesicle, VS ventral sucker, *papilla*
Fig. 18 A  *D. breviductus* camera lucida drawing

B  Micrograph (phase contrast) showing prosoma

AT anterior testis, E ecsoma, IC intestinal caecum, P prosoma, PH pharynx, PP pars prostatica, PT posterior testis, OS oral sucker, OV ovary, SO sinus organ, SV seminal vesicle, VS ventral sucker, UT uterus, VT vitellarium
Fig. 19 A  *D. ivanosii* : Camera lucida drawing

B  Micrograph of *D. ivanosii* (stained with borax carmine)

AT anterior testis, EC esoma, IC intestinal caecum, OO ootype, OV ovary, OS oral sucker, P prosoma, PH pharynx, PP pars prostatica, PT posterior testis, SS sinus sac, SV seminal vesicle, UT uterus, VS ventral sucker, VT vitellarium
Fig. 20 A  Micrograph of fore body of *D. ivanosi* showing elongated pharynx (stained with borax carmine)
B  Micrograph of fore body of *D. ivanosi* showing plications & excretory ducts (borax carmine)
C  Micrograph of *D. ivanosi* showing testes (stained with borax carmine)
D  Camera lucida drawing of seminal vesicle
E  Micrograph of *D. ivanosi* showing ovary & vitellarium (stained with borax carmine)

AT anterior testis, DL distal lobe, ML middle lobe, OS oral sucker, OV ovary, PH pharynx, PL proximal lobe, PP pars prostatica, PT posterior testis, SV seminal vesicle, VS ventral sucker, ⚪ excretory duct, ☕ vitellarium
DISCUSSION

The distinguishing features of the eight species studied are given in Table 8. *D. hippuri* roughly resembled *D. longisinus* and *D. breviductus* in their general morphology with the unaided eye. *D. hippuri* differed from both in having a short body and relatively similar length of prosoma and ecsoma (Rekha & John, 2002a). Slender elongated body was characteristic of *D. longisinus* and *D. breviductus*, while the shorter ecsoma in *D. breviductus* made it distinct from the long ecosmate *D. longisinus*. *D. tornatus*, the first recorded species of this genus from dolphin fishes, shows an apparent resemblance to *D. coryphaenae* and *D. barbatus*. *D. barbatus* was first described by Cohn (1902) as *Lecithocladium barbatum*, and later re-described and placed in the genus, *Dinurus*, by Looss (1907, 1908). Since that time, except for the work of Vigueras (1958), and Gibson (1976) no further information of this species was available. The presence of long integumentary outgrowths in the fore body of *D. barbatus* differentiates it from other *Dinurus* species. These outgrowths are morphologically different from the plications. Further studies are required to confirm the structural details and functional significance of these outgrowths. *D. tornatus* resembles *D. coryphaenae* in having prominent prosomal plications, stout body and also in the sucker ratio. The shorter ecsoma of distinguishes *D. coryphaenae* from *D. tornatus*, in which the ecsoma has double the length of prosoma. Though Rudolphi (1819) first reported *D. tornatus*, a detailed description of this species was made by Looss (1907, 1908), Dollfus
(1927) and Siddiqi & Cable (1960). According to Gibson, the length of *D. tornatus* varied from 2 to 15 mm.

*D. ivanosi* possesses certain characters different from all the other known species of *Dinurus*. The presence of distinct prosoma and ecsoma, smaller size of the oral sucker compared to the ventral sucker and pre-ovarian testes may be cited to justify the inclusion of this species under the family Hemiuridae. Because of the plicated prosoma, trilobed seminal vesicle, the smaller size of the ovary than the testes and vitellaria with seven narrow tubes, this species is included under the subfamily Dinurinae. Moreover, like any other *Dinurus* species, it also has been recovered from the stomach of dolphin fish. The subfamily Dinurinae generally has a globular pharynx smaller than the oral sucker (Yamaguti, 1971), but in *D. ivanosi* the pharynx is elongated. Bray (1990) reported an elongated pharynx in *Lecithocladium angustiovum* (subfamily: Elytrophallinae of Hemiuridae). But the genus *Lecithocladium* differs from *Dinurus* in numerous aspects, the prominent being the larger size of the oral sucker and the uni-lobed seminal vesicle (Gibson, 2002). *D. ivanosi* differs from the other *Dinurus* species in the possession of a larger anterior testis. Unlike other species, *D. ivanosi* has an oblong seminal vesicle with a small distal lobe. The unique characters of *D. ivanosi*, like the elongated pharynx, larger anterior testis, small distal lobe of the seminal vesicle, short and slender vitellaria and the smaller egg size, were sufficient to warrant recognizing it as a new species (Rekha & John, 2004).
The prosoma of all the eight species reported in the present study is plicated while ecsoma devoid of plications. This is in agreement with the observations of Yamaguti (1971). However, Nadakal et al. (1991) reported the presence of plications on the ecsoma of *D. hippuri*. This observation might have been an oversight and the present author could not observe plications even in scanning electron micrographs (Chapter 3). Furthermore, the distribution of prosomal plications in *D. hippuri* was not uniform unlike the previous reports. The anterior limit was far below the ventral sucker (Rekha & John, 2002a). Bray et al. (1993) reported the absence of plications on anterior prosoma up to gonad level in *D. longisinus*. But the present author could observe plications up to posterior rim of oral sucker as in *D. breviductus* and this is in agreement with the observations of Gibson (1976). In *D. scombri* plications were distributed from below the ventral sucker level as reported by Léon-Régagnon et al. (1997) while in *D. tornatus* they were found throughout the prosoma as illustrated by Yamaguti (1971). Similar arrangement was observed in *D. ivanosi, D. barbatus* and *D. coryphaenae*.

Sucker ratio was 1: less than 2 in *D. hippuri, D. ivanosi, D. coryphaenae* and *D. tornatus*, while in *D. longisinus, D. breviductus* and *D. barbatus* it was 1:2 or a little more. Bray (1990) and Bray et al. (1993) in their reports specified the sucker ratio of *D. longisinus* as 1:1.75 - 1.88 and 1:1.82 respectively. In the present study the sucker ratio of *D. longisinus* was found to be 1:2 or a little more, in all the samples studied. Hence it is concluded that the specimens studied by the above authors were not *D. longisinus*. Similar size of the oral and ventral suckers was
characteristic of *D. scombri* (Yamaguti, 1934, Leon-Regagnon *et al.*, 1997). The acetabular papillae in *D. hippuri* (mentioned in Chapter 3) are absent in other *Dinurus* species (Rekha & John, 2002a). Baldwin (1995) and Fairweather *et al.* (1999) reported similar papillae in *Allocreadium lucyae* (Digenea: Allocreadiidae) and *Fasciola hepatica* (Digenea: Echinostomata) respectively. Haas *et al.* (1997) reported an acetabular gland in *Schistosoma mansoni* cercariae. Histological and histochemical studies are needed to confirm their glandular nature.

This is the first report on a new host of *D. scombri*. *D. scombri* has been reported previously from *Scomber japonicus* (Yamaguti, 1934), *Euthynnus alletteratus* (Manter, 1947), *Decapterus sanctahelenae* and *Selar crumenophtalmus* (Leon-Regagnon *et al.*, 1997). This is also the first report of *D. scombri*, and *D. tornatus*, *D. barbatus*, *D. breviductus*, *D. coryphaenae* and *D. longisinus* from India. All the morphological and anatomical features of *D. scombri* observed in the present study, were similar to those reported by Yamaguti (1934). *D. euthynni* was first reported as a new species by Yamaguti (1934) from *Euthynnus pelamys* (Linn.), the skip jack tuna of the Pacific because of the larger size of ecsoma. *E. pelamys* and *C. hippurus* were reported to overlap in their distribution in the Pacific seas at the same seasons – 58° N to 47° S and 180° W and 180° E (Collette & Nauen, 1983). Hence the possibility of an accidental infection of *D. scombri* to *E. pelamys* can not be ruled out.

The male reproductive organs of *Dinurus* were found confined only to prosoma. Testis in *Dinurus* is paired and generally globular, but oval in *D.*
*longisinus* and sub-globular in *D. tornatus*. In *D. barbatus* the testes overlap, but in *D. hippuri, D. scombri* and *D. coryphaenae*, they are contiguous. In *D. breviductus* posterior testis is placed far behind the other, while that of *D. tornatus* remained oblique. The cone shaped structure at the junction between each testis and vas deference was found to be unique to *D. breviductus*. Bray et al. (1993) reported that lobed nature of seminal vesicle was clearly visible only when it was empty. But filling and emptying of seminal vesicle may not cause any variations in the nature and number of lobes. Improper staining process may, sometimes, mask the clarity of seminal vesicle so that the partition may not be clear. Partitions may not clear in thick walled seminal vesicle, but the longitudinal sections show the exact nature. Trilobed seminal vesicle was present in all the *Dinurus* species except *D. scombri*, in which it is four lobed. Oblong seminal vesicle with large middle lobe is characteristic of *D. longisinus* while that in *D. hippuri* and *D. breviductus*, stout with small middle lobe (Rekha & John, 2003). In *D. barbatus* and *D. coryphaenae* the three lobes were of same size.

The length of pars prostatica and sinus sac determines the position of the seminal vesicle. Very short pars prostatica and sinus sac placed the seminal vesicle of *D. barbatus* and *D. scombri* immediately behind the ventral sucker. Long pars prostatica was characteristic of *D. longisinus* and *D. breviductus*. The length and convolutions of pars prostatica of *D. hippuri* were almost similar to that of *D. tornatus, D. coryphaenae* and *D. ivanosi*. Large prostatica cells and fixed turns of the pars prostatica were characteristic of *D. tornatus*. Pars prostatica was glandular
throughout its length in *D. hippuri* and *D. tornatus*, while in others, the two ends remained non-glandular. The size, shape and position of sinus sac were also different. In *D. scombri*, *D. coryphaenae*, *D. breviductus*, *D. ivanosi* and *D. barbatus* the sinus sac was far anterior to the ventral sucker while that in *D. tornatus* and *D. longisinus* reached about half the level of ventral sucker. In *D. hippuri* the lower limit of sinus sac was behind the ventral sucker.

In *D. tornatus* common hermaphroditic duct for sperm duct and metatrem passed through the sinus sac. This feature was reported in all the *Dinurus* species by Yamaguti (1971) and Oliva (1984). In the histological studies it is observed that in *D. hippuri* the sinus sac bears separate sperm duct and metatrem (Chapter 6). Elongated sinus organs were characteristic of *D. hippuri*, *D. breviductus* and *D. longisinus*, but short or indistinct in other species. In *D. tornatus* the part of sinus sac bearing sinus organ appeared to have a muscular sphincter. This is the first report of such a sphincter in the genus *Dinurus*. Reid et al. (1966) reported the presence of two sphincters associated with the sinus sac in *Erilepturus tiegsi* (Hemiuridae). Nadakal et al. (1991) in their report on *D. hippuri* illustrated the position of genital apertures below the pharynx. In the present study, as in the case of other *Dinurus* species, the position of genital apertures was found just below the oral sucker ventrally. Digenean trematodes generally have a common genital opening for both male and female reproductive ducts. But in some it can be separate (Noble & Noble, 1973). According to Gibson (2002) all the *Dinurus* species reported till then had a common genital aperture. Separate male and female
genital pores were reported in *D. hippuri* (Chapter 3 & 6) by Rekha and John (2002a & 2003). Distinct cirrus was clearly visible in *D. hippuri* and *D. tornatus*, smooth in *D. hippuri* while, papillated in *D. tornatus*.

The female reproductive organs were confined to the prosoma, except a portion of uterus and the backwardly running vitellaria. Single post testicular ovary is characteristic of all the 8 *Dinurus* species. Leon-Regagnon et al. (1997) reported bilobed ovary in *D. scombri*. This is certainly a misinterpretation. The ootype attached to the lower margin of ovary might have been mistaken as another lobe of ovary. Review of literature shows no convincing report of a bilobed ovary in *Dinurus*. Lobulation of the ovary was reported as a specific character of the genus *Stomachicola* of the family Hemiuridae (Pande et al., 2000; Gibson, 2002). According to Gibson (2002), single post-testicular ovary is characteristic of even the subfamily Dinurinae.

Distribution and nature of vitellaria differ in most species even though the number (7) is constant in all. In *D. barbatus* and *D. scombri* vitellaria were restricted to the prosoma, but its others the backwardly directed vitellaria extended to the ecsoma. In *D. longisinus* and *D. tornatus* three of them extended forward (Rekha & John, 2002a), and only two in *D. hippuri*, *D. coryphaenae*, *D. breviductus*, *D. ivanosi* and *D. barbatus*. In *D. scombri* the uterine coils masked the vitellaria and hence difficult to observe the actual disposition.

Vitelline field determines the nature of egg produced by the worm. Thick shelled and large eggs were produced by the worms with long, thick vitellaria. In
D. hippuri, D. longisinus D. breviductus and D. tornatus large vitelline field was observed. These worms show larger eggs compared to the rest. Smallest size of egg was reported in D. scombri, while the largest in D. breviductus. The size of uterus found to determine the number of eggs lodged. Number of egg is high in D. tornatus. The descending limb of uterus in all the Dinurus species extended to more than half of ecsoma, except D. scombri, in which it extend only to one fifth of the ecsomal length.

The eight species reported, described and discussed here in this chapter are distinct, each with its own identity and species status. Molecular approaches, including DNA profiling, may provide valuable information on the evolutionary status and kinship of the different species. Vilas et al. (2002) used electrophoresis to differentiate three sympatric species of Lecithochirium (Hemiuridae) by enzyme analysis.
Parasitic infection of a host by more than one species belonging to the same genus is a rare and such a phenomenon was observed in the present study. Eight species of *Dinurus* were isolated from the stomach of the dolphin fish *C. hippurus*. They were *D. hippuri, D. barbatus, D. longisinus, D. scombri, D. coryphaenae, D. breviductus, D. ivanosi* and *D. tornatus*. The adult worms were studied in detail for their taxonomic identity and found variations in the arrangement of tegumental plications, nature of seminal vesicle, sinus sac, sinus organ and the sucker ratio. The tubular integumentary processes below the oral sucker appear to be a character specific to *D. barbatus*; while the quadripartite seminal vesicle and equal sized suckers were specific to *D. scombri*. In the possession of six acetabular papillae, an acetabular prominence and eversible smooth cirrus *D. hippuri* stands distinct. The disposition of sinus sac at the mid level of ventral sucker, three forwardly directed vitellaria and the long pars prostatica are characteristic to *D. longisinus* while the folded pars prostatica, large prostatic gland cells, single median excretory duct and papillated cirrus make *D. tornatus* distinct. *D. breviductus* was distinct in having large seminal vesicle and a cone shaped elevation of testes, while *D. coryphaenae* in having short sinus sac and a sucker ratio 1:51. The most important diagnostic features of the recently reported species *D. ivanosi* are the general shape and proportion of the body, elongated pharynx and large anterior testis.