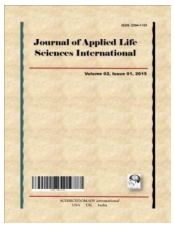

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A Morphological Study of *Kroyeria minuta* Pillai, 1968 (Copepoda: Eudactylinidae) Infecting the Milk Shark, *Rhizoprionodon acutus* Rüppell, 1937 off Nellore Coast, Bay of Bengal, India

C. Srinivasa Kalyan[1] and V. Anuprasanna[1*]

[1]Department of Zoology, Yogi Vemana University, Kadapa, 516 003, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author CSK had collected the host samples from the sampling sites, carried out dissections and collected parasites for further processing, involved in data compilation and literature survey while author VA is the corresponding author who prepared the initial manuscript, framed and formatted to get a final manuscript.

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ABSTRACT

Copepods of the genus *Kroyeria* Van Beneden, 1853 (Eudactylinidae) are ectoparasites of Elasmobranchs frequently infecting the gill filaments. Eudactylinid parasites were sampled from 152 milk sharks, *Rhizoprionodon acutus* collected from the Nellore coast, Bay of Bengal. With the application of scanning electron microscopy (SEM) and light microscopy we identified *Kroyeria minuta* Pillai, 1968, which is a new geographical record for the east coast of India. Fifteen parasites were acquired from the gill filaments of 9 infected fishes and its main morphological characteristics include minute structure, long dorsal stylets not extending beyond the hind border of the 4th thoracic segment, thoracic segments with interpodal bars, short 3-segmented abdomen, uniseriate

**Corresponding author: E-mail: annuprasanna@gmail.com;kalyan.sreenivas02@gmail.com.*

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egg sacs, 8-jointed antennule, robust antenna, slender maxilla with apically forked claw and two segmented maxillipede.

Keywords: Ectoparasites; Kroyeria minuta; Rhizoprionodon acutus; gill filaments; Bay of bengal; SEM.

1. INTRODUCTION

Rhizoprionodon acutus Rüppell, 1937 is a cartilaginous fish included in the class Elasmobranchii. They are primarily marine organisms widely distributed in tropical, subtropical and temperate regions. The body of the shark is elongate, fusiform and head is dorsoventrally flattened. Generally they grow up to 2-8 feet length. These sharks are carnivorous fish its diet consists of fish of all kinds, sea birds, turtles, etc. Sharks, as top predators, serve as a suitable host for the parasitic crustaceans. The parasitic crustaceans are the major group of parasites inhabiting the fish of marine, brackish and fresh waters. Most of the parasitic crustaceans belong to Isopoda, Branchiura and Copepoda [1-6]. The genus *Kroyeria* (Copepoda: Eudactylinidae) is the largest of the three genera of the family Kroyeriidae with 21 nominal species infecting the about 200 species of ground sharks [7-12]. Most *Kroyeria* species are host specific on carcharhiniform sharks of the families Carcharhinidae and Triakidae with a few species having been reported from the family Sphyrnidae [13,14]. Gill filaments and gill arches of elasmobranchs serve as excellent sites for anchoring because they are sources of nutrients for ectoparasites [15]. *Kroyeria* is the second most species-rich genus of all parasitic copepods inhabiting the secondary lamellae of the gills by using the chelate antennae as primary attachment organs assisted by the dorsal stylets, interdorsal stylets and maxillipeds which serve as secondary attachment organs [13-14,16]. Except the female of *K. caseyi* Benz and Deets, 1986, which is a mesoparasite of the genus, *Kroyeria* is found entrenched into the interbranchial septa of the host species, whereas rest of the females and males of the other species in the genus are ectoparasites (Deets,1994). Moreover, *Kroyeria* species display sexual dimorphism with males being smaller than females mainly because of the long, tubular genital complex of the female [17]. While from the west coast of India (Kerala coast) four species of *Kroyeria* are reported: *Kroyeria echinata* Rangnekar, 1956; *K. elongata* Pillai, 1967; *K. minuta* Pillai, 1968 and *K. sphyrnae* Rangnekar, 1957 by Pillai [18], there are no such studies from the east coast of India. In the present survey, an attempt was made to analyze the parasitic fauna of the elasmobranchs off Nellore coast, Bay of Bengal and *K. minuta* was reported for the first time from this coast. Although SEM (Scanning electron microscopy) studies on copepod parasites of fishes are still rare [8,19-22], we employed both scanning electron microscopy and light microscopy to precisely describe the morphological characteristics of *K. minuta*.

2. MATERIALS AND METHODS

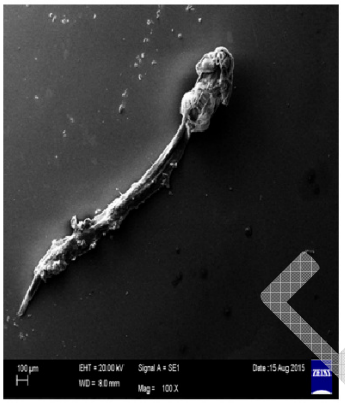
We examined 152 *Rhizoprionodon acutus* procured from the local fisherman and nearby fish markets were examined for the ectoparasites during the study period from January 2014 to December 2015. These parasites were found attached to gill filaments of the host and no propensity for specific gills was observed. Collected specimens were temporarily preserved in 10% formaldehyde. The parasites observed and identified under the Lynx Trinocular microscope (N-800M) by capturing the microphotographs and line diagrams were drawn with the aid of an attached drawing tube. Ocular micrometer measurements were in millimeters unless otherwise indicated. For SEM specimens, copepod parasites were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at 4℃ for 1 hour. They were washed in the same buffer before post-fixation in 1% osmium tetroxide in the same buffer at 4℃ for 1 hour. Specimens were dehydrated through a graded series of ethanol (70%-100% at 5-10 min interval), critical point dried and sputter coated with gold. SEM photographs at various magnifications were captured with a Carl Zeiss Scanning electron microscope (ΣIGMA TM) facility at Sri Venkateswara University, Tirupati. Identifications were made according to Kabata (1979) and Pillai (1985).

3. RESULTS

SEM study facilitates the comprehensive morphological identification of *K. minuta*, even to the species level. The structure of the copepod is shown in detail with SEM photographs (plate-1; Figs. 1 to 6) and the line diagrams (Plate-2; Figs. 1-15).

Plate-1

1



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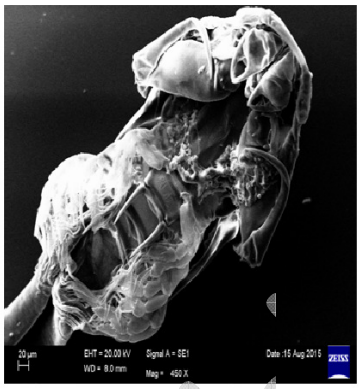

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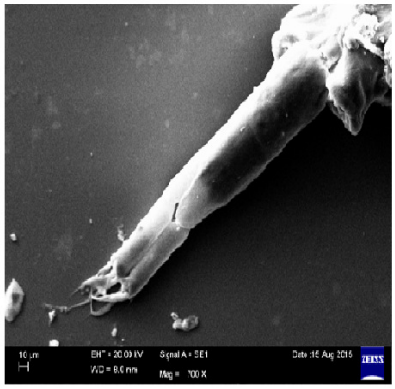

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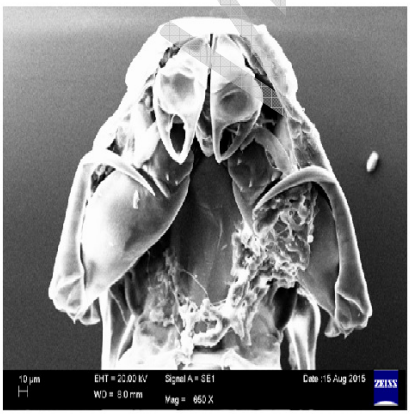

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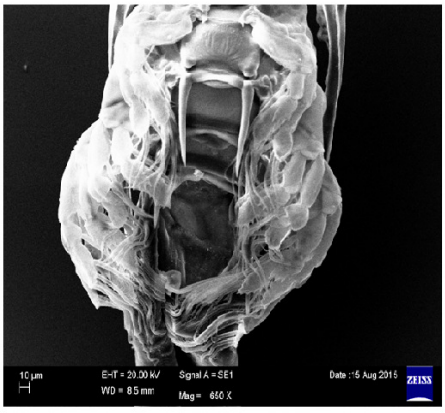

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Plate 1. Fig. 1. Female of *Kroyeria minuta* (Original light microscope) 40X, 2. Female of *K. minuta* (Original SEM) 100X, 3. Cepahalothorax of *K. minuta* 450X, 4. Abdomen and caudal rami of *K. minuta* 700X, 5. Mouthparts of *K. minuta* 650X, 6. Legs I-IV of *K. minuta* 650X

Table 1. A review of *Kroyeria* species, their distinguishing features, reported hosts and their geographical locations [13,16,10,11]

***Kroyeria* species**	**Distinguishing characteristics**	**Host species**	**Geographical range**
Kroyeria branchiocetes Deets, 1994	Similar in appearance to *K. cresseyi*, *K. lineata*, *K. rhophemophaga* and *K. triakos* in that the antenna has a claw with only two slender setae. However, differs from these species in that it possesses endopodal denticulations on the second and third segments of all the swimming legs (Deets 1994).	*Carcharhinus amblyrhynchos* (Bleeker, 1856) (Deets 1994).	Red sea (Deets 1994).
Kroyeria brasiliense Thatcher & Júnior, 2006	Similar to *K. deetsi* but differs from it in the following: larger maxillipeds which project well beyond the lateral margins of the cephalothorax; shorter, two-segmented abdomen in contrast to the long, slender, three-segmented abdomen in *K. deetsi*; rounded second and third endopodal segments of equal length and are devoid of denticles, in contrast to the length of the third endopodal segments which are twice or more than twice as long as those of the corresponding second endopodal segments (Dippenaar *et al*. 2000; Thatcher & Júnior 2006).	*Galeorhinus galeus* (Linnaeus, 1758) (Thatcher & Júnior 2006).	Atlantic Ocean near Rio Grande, Brazil (Thatcher & Júnior 2006).
Kroyeria carchariaeglauci Hesse, 1879	Characterized by the combination of bifid dorsal stylets; caudal rami with stout, pyriform, pinnate setae adjacent to the two elongate pinnate setae in females as well as the relative length to width ratio of the caudal ramus of the male (Deets 1994).	*Prionace glauca* (Linneaus, 1758); *Carcharhinus falciformis* (Müller and Henle, 1839); *Carcharhinus longimanus* (Poey, 1861); *Carcharhinus plumbeus* Nardo, 1827; *Carcharhinus leucas* (Müller and Henle, 1839) (Deets 1994; Dippenaar 2005; Dippenaar & Jordaan 2007). Questionable records have been provided from *Eulamia sp.*, *Mustelus asterias* Cloquet, 1819, *Mustelus mustelus* (Linnaeus, 1758) and *Squalus blainville* (Risso, 1827) (Deets 1994).	Eastern North Atlantic, Mediterranean, Japanese waters, Chile, Western North Atlantic, Tunisian waters, Eastern North Pacific, Mexico, Channel Islands, Southern California Bight, Madagascar (Deets 1994;), East coast of South Africa (Dippenaar 2005; Dippenaar & Jordaan 2007).
Kroyeria caseyi Benz and Deets, 1986	Largest and only known mesoparasitic *Kroyeria* species. It is characterized by a genital complex that is extremely elongate, forming 95% of the entire body; maxilla with very elongate claw; antenna aperture with reduced seta; inflated abdomen with only one segment; caudal rami lacking the typical medial fringe of setules but bearing stout naked setae (Deets 1994).	*Carcharhinus signatus* (Poey, 1868) (Deets 1994).	Western North Atlantic (Deets 1994).
Kroyeria cresseyi Deets, 1994	Similar to *K. branchiocetes*, *K. lineata*, *K. rhophemophaga* and *K. triakos* by possessing antennae with the claw having only two slender setae. However, differs from *K. branchiocetes* and *K. lineata* because the claw and the corpus of the antennae lack the large membranous expansion distally. It differs from *K. triakos* by having the terminal segment of the third exopod possessing only four pinnate setae, one lateral slender seta with serrated membrane and one lateral semi-pinnate seta (*K. triakos* has five pinnate setae and one slender naked seta). Contrary to *K. rhophemophaga* which has a subquadrangular cephalothorax, *K. cresseyi* has an orbicular cephalothorax. Additionally, *K. cresseyi* has a bifid dorsal stylet as opposed to the other species that are similar to it (Deets 1994).	*Triakis semifasciata* Girard, 1855 (Deets, 1994)	Inshore waters off El Segundo, Seal Beach and Palos Verde, California (Deets 1994).

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***Kroyeria* species**	**Distinguishing characteristics**	**Host species**	**Geographical range**
Kroyeria decepta Deets, 1994	Very similar to *K. carchariaeglauci*, but differs by possessing pinnate setae (naked in *K. carcharhiaeglauci*) on endopod of maxillule, pectinate lateral membranes (thin and smooth in *K. carcharhiaeglauci*) on second and third segments of exopod of leg 1 and teeth of alternating sizes on the mandible (uniform size in *K. carcharhiaeglauci*). *K. decepta* is bigger than *K. carcharhiaeglauci* in size (Deets 1994).	*Carcharhinus obscurus* (Lesueur, 1818) (Deets 1994; Dippenaar & Jordaan 2007).	West coast of Florida, tropical Northeastern Pacific (Deets 1994), East coast of South Africa (Dippenaar & Jordan, 2007)
Kroyeria deetsi Dippenaar, Benz & Olivier, 2000	Characterized by the third endopodal segments of legs 1–4 which are about twice (leg 1, 2 and 4) or more than twice (leg 3) as long as the corresponding second endopod segments (Dippenaar *et al*. 2000).	*Carcharhinus brevipinna* (Müller and Henle, 1839) (Dippenaar *et al*. 2000).	East coast of South Africa (Dippenaar *et al*. 2000; Dippenaar 2005).
Kroyeria dispar Wilson, 1935	Characterized by the unusually wide cephalothorax; lack of endopodal denticulations; two elongate, pinnate setae on the medial margin of endopods of legs 1 and 2; maxillule with spinulated endopod and maxilliped having peculiar cuticular flaps on myxal area (Deets 1994).	*Galeocerdo cuvier* (Péron & Lesueur, 1822) (Deets 1994; Dippenaar & Olivier 1999).	West coast of Florida (Deets 1994), East coast of South Africa (Dippenaar & Olivier 1999; Dippenaar 2005).
Kroyeria echinata Rangnekar, 1956	Dorsal stylets short and stout, resembling those of *K. dispar* and *K. papillipes*, but smaller. The presence of endopodal denticulations on the second endopodal segment of all the legs is the same in both *K. echinata* and *K. papillipes*, but all the endopods of *K. dispar* lack denticulations. *K. papillipes* differs from *K. echinata* by having an orbicular cephalothorax and different armature on the caudal rami as well as on the legs (Deets 1994).	*Sphyrna zygaena* (Linnaeus, 1758) (Deets 1994).	Indian Ocean (Deets, 1994)
Kroyeria elongata Pillai, 1967	Very elongated claw and corpus of antenna (Deets 1994).	*Rhizoprionodon acutus* (Rüppell, 1837) and *Carcharhinus sorrah* (Müller and Henle, 1839) (Deets 1994).	Indian Ocean (Deets 1994).
Kroyeria gemursa Cressey, 1967	The distal region of the last segment of abdomen is laterally bulging and heavily sclerotized; antenna with thickened claw and extension of the corpus thickened resulting in reduced aperture; labrum with large patches of spinules on distolateral surfaces; second and third segments of leg 1 and 2 with numerous (25–33)	*Sphyrna mokarran* Rüppell, 1837 (Deets 1994; Dippenaar 2005; Dippenaar & Jordaan 2007).	Madagascar, Indian Ocean, West coast of Florida, (Deets 1994; Dippenaar 2005), East coast of South Africa (Dippenaar 2007).
Kroyeria lineata van Beneden, 1853	Endopods devoid of endopodal denticulations, proximal region of the claw of the antenna bearing only two prominent setae, the only *Kroyeria* species with distal membranous extensions near the tip of the claw of the maxilla (Deets 1994).	*Galeorhinus galeus* (Linneaus, 1758), *Mustelus mustelus* (Linneaus, 1758), *Mustelus asterias* Cloquet, 1821 and *Mustelus punctulatus* Risso, 1827. Other records which are questionable come from hosts like *Sphyrna zygaena* (Linnaeus, 1758), *Carcharhinus limbatus* (Müller and Henle, 1839), *Negaprion brevirostris* (Poey, 1868) and *Prionace glauca* (Linneaus, 1758) (Deets 1994).	Adriatic Sea, North Sea, Mediterranean off Tunisia, Japan (Deets 1994).

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***Kroyeria* species**	**Distinguishing characteristics**	**Host species**	**Geographical range**
Kroyeria longicauda Cressey, 1970	Characterized by a deeply incised, bifid dorsal stylet with lateral tine; caudal rami with lateral cuticular flange and the small number of unusually large endopodal denticulations (Deets 1994).	*Carcharhinus limbatus (*Müller & Henle, 1839), *Carcharhinus brevipinna* (Müller and Henle, 1839) (Deets 1994).	Florida, Mozambique Channel (Deets 1994; Dippenaar 2005), East coast of South Africa (Dippenaar & Jordaan 2007).
Kroyeria minuta Pillai, 1968	Characterized by small size; dorsal stylets that are long and bifid, extending to the posterior margin of the fourth 30 thoracic segment; serrated medial margin; lateral margin of coxa of the second leg with atypical patch of spinules; third segment of exopod of leg 2 with six pinnate setae (Deets, 1994)	*Rhizoprionodon acutus* (Rüppell, 1837) (Pillai 1968).	Indian ocean (Pillai 1968
Kroyeria papillipes Wilson, 1932	Distinguished by being the only known *Kroyeria* species having all six setae on the caudal rami being elongate and pinnate (Deets 1994).	*Galeocerdo cuvier* (Péron & Lesueur, 1822) (Deets 1994; Dippenaar & Jordaan 2007).	West coast of Florida (Deets 1994), East coast of South Africa (Dippenaar & Jordaan 2007).
Kroyeria procerobscena Deets, 1994	Unusually long genital complex which forms 80% of the entire body length; the proximal region of the bifid dorsal stylet with a unique lateral tine; caudal rami with two elongate, proximally inflated medially-pinched pinnate setae (Deets 1994).	*Carcharhinus leucas* (Müller and Henle, 1839) and *Carcharhinus amboinensis* (Müller & Henle, 1839) (Deets 1994; Dippenaar 2005; Dippenaar & Jordaan 2007).	Mozambique Channel, (Deets 1994; Dippenaar 2005), East coast of South Africa (Dippenaar & Jordaan 2007).
Kroyeria rhophemophaga Deets, 1994	Similar *to K. branchiocetes*, *K. lineata*, *K. cresseyi* and *K. triakos* by possessing antennae with the claw bearing only two slender elongate setae, rather than three. However, differs from *K. branchiocetes* and *K. lineata* because the claw and the corpus of the antennae lack the large membranous expansion distally. It differs from *K. triakos* by having the terminal segment of the third exopod possessing only four pinnate setae and two lateral slender setae. Contrary to *K. cresseyi which* has an orbicular cephalothorax, *K. rhophemophaga* has a subquadrangular cephalothorax (Deets 1994).	*Galeorhinus galeus* (Linnaeus, 1758) (Deets 1994).	New Zealand, Eastern Atlantic, Eastern North Pacific (Deets 1994).
Kroyeria spatulata Pearse, 1948	Characterized by the presence of unique sinuous, pectinate membranes, located medial to the short, spiniform setae on all segments of the exopod of leg 4 and wrapping down the lateral margins of the second and terminal segments (Deets 1994).	*Rhizoprionodon terraenovae* (Richardson, 1836), *Carcharias littoralis* (Rafinesque, 1810), *Carcharhinus limbatus* (Müller & Henle, 1839), *Rhizoprionodon acutus* (Rüppell, 1837), *Negaprion brevirostris* (Poey, 1868) and *Carcharhinus sorrah* (Müller and Henle, 1839) are errors (Deets 1994). *Carcharhinus leucas* (Müller and Henle, 1839). Reports of *K. spatulata* on the *Carcharhinus brevipinna* (Müller and Henle, 1839) and *Carcharhinus sorrah* (Müller and Henle, 1839) are errors (Deets 1994).	North Carolina, Bahamas, Gulf of Mexico, Indian Ocean, West coast of Florida (Deets 1994).

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***Kroyeria* species**	**Distinguishing characteristics**	**Host species**	**Geographical range**
Kroyeria sphyrnae Rangnekar, 1957	Characterized by long, acute, lissome dorsal stylets, seven-toothed mandible formula, relatively short interpodal stylets which barely reach the distal margin of the basipods of legs 2–4 (Deets 1994).	*Sphyrna zygaena* (Linnaeus, 1758) and *Sphyrna lewini* (Griffith and Smith, 1834) (Deets 1994; Dippenaar *et al.* 2001). Additionally, reports of *Kroyeria sphyrnae* on *Chiloscyllium punctatum* Müller and Henle, 1838 and *Carcharhinus acronotus* (Poey, 1860) are questionable (Deets 1994).	India, Australia, West coast of Florida, Hawaiian Islands, Southern Sea of Cortez, Mexico, Eastern North Pacific, Indian Ocean (Deets 1994; Dippenaar 2005), East coast of South Africa (Dippenaar, *et al.* 2001; Dippenaar & Jordaan 2007).
Kroyeria sublineata Yamaguti and Yamasu, 1959	Resembles *K. lineata* in appearance but differs from it in the chaetotaxy of the legs. The number of setae and spines on the terminal segments of exopods of legs 1–4 in *K. sublineata* is 6,6,6,6 but 6,7,7,7 in *K. lineata* (Izawa 2008).	*Mustelus manazo* Bleeker, 1855 and *Mustelus griseus* Pietschmann, 1908 (Deets 1994; Izawa 2008).	Inland Sea of Japan Tanabe Bay (Deets 1994; Izawa 2008).
Kroyeria triakos Fukui, 1965	The only *Kroyeria* species with the terminal exopodal segment of leg 3 bearing five elongate, pinnate setae. Additionally, the first and second segments of the fourth exopod lack typical lateral setae (Deets, 1994).	*Triakis scyllium* Müller and Henle, 1839 (Deets 1994).	Japan (Deets 1994

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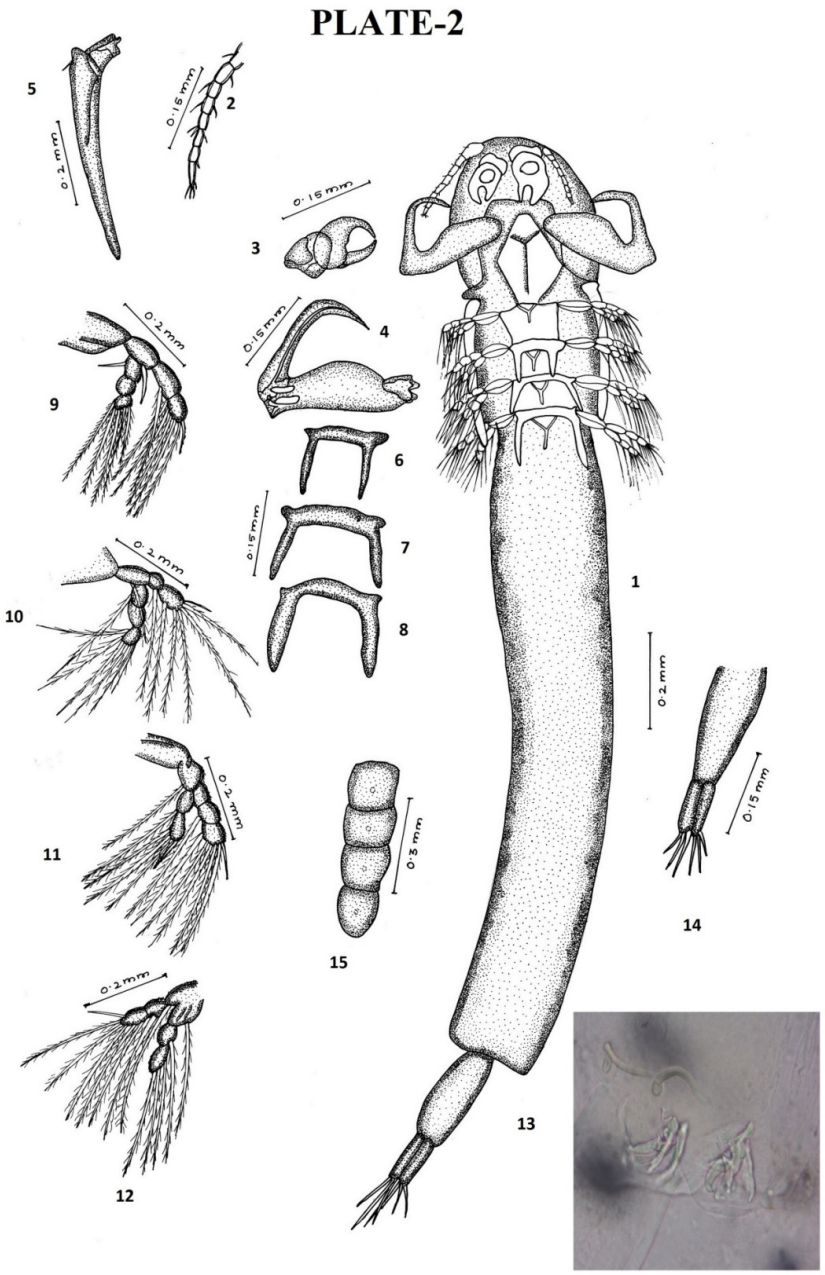

Plate 2. Fig. 1. Female of *Kroyeria min*uta- Ventral view, 2. 1st Antenna 3. 2nd Antenna, 4. Maxillipede, 5. Dorsal stylet, 6, 7, 8: Interpodal bars, 9, 10, 11, 12: Legs I-IV, 13. Leg-V (100X) (Original light microscope), 14. Abdomen and Caudal rami, 15. Uniseriate egg sac

3.1 *Kroyeria minuta* Pillai, 1968 (Plate-2; Fig. 1-15)

Family: Eudactylinidae

Genus: *Kroyeria*

Kroyeria minuta Pillai, 1968

Female *Description*: (Based on 4 specimens, measurements in mm): Body elongated and slender (2.46-3.1 × 0.22-0.25) in length (Plate-1, Fig. 1). Cephalothorax (0.2-0.57 × 0.19-0.25) broader than long (Plate-1, Fig. 2). Postero-lateral lobes small, triangular and remains apart from dorsal Stylets (Plate-1, Fig. 3). Dorsal stylets long (0.28-0.3 × 0.02) apically forked

reaching up to the hind border of the fourth thoracic segment, forked apically. Thoracic segments sub-equal to 3 interpodal bars (I-0.09-0.1 × 0.07-0.08; II-0.13-0.15 × 0.13; 0.12-0.18 × 0.13). Genital segment (0.55-0.75 × 0.06-0.08) three times as long as body in front. Egg sacs uniseriate. Abdomen short, slightly 3-segmented, first and second abdominal segments are more similar in shape than third. Caudal rami (0.25-0.3 × 0.1) with one long seta and two long spines (Plate-1, Fig. 4). Antennule 8-jointed with 13 setae. Antenna robust, chelate with stout prehensile claw and thumb like chela, claw with prominent inner distal spine along the inner distal margin place within the groove. Maxilla slender, claw apically forked. Maxillipede two segmented, basal segment slightly bulging in the middle and distal segment, with a row of tubercules (Plate-1, Fig. 5).

Legs 1 to 4 biramous, rami three-segmented (Plate-1, Fig. 6). Leg 5 reduced to three setose and one naked seta, located halfway along the genital complex (Plate-1, Fig. 7).

Armature of legs as follows (Roman numerals indicate spines and Arabic numerals indicate setae):

	Exopod	Endopod
Leg-I	I-0; 0-1; I-5	I-0; 0-0; 0-5
Leg-II	0-1; 0-1; I-5	0-1; 0-0; 0-6
Leg-III	0-1; 0-1; I-5	0-1; 0-0; 0-5
Leg-IV	0-1; 0-1; I-5	0-1; 0-1; 0-3

3.2 Taxonomic Summary

Host: *Rhizoprionodon acutus* Rüpell, 1937

Locality: Nellore coast, Bay of Bengal, India.

Site of infestation: gill filaments.

Record of specimens: 15 (All females)

No. of fishes examined: 152

No. of fishes infected: 09; Prevalence (%): 5.92

4. DISCUSSION

A detailed review of *Kroyeria* species, their distinctive features, reported hosts and their geographical locations is described [13,16,10,11] (Table 1). The species described in the present paper was identified as *Kroyeria minuta* because it showed the distinctiveness described by Pillai (1968) in the original description. The use of SEM enabled to supplement previous descriptions with more accurate details. The structure of abdomen described by Pillai (1985) is single segmented whereas in the SEM studies, the abdomen is short, slightly 3-segmented, first and second abdominal segments are similar than that of third segment. The structure of antennule, antenna, maxilla, maxillipede, thoracic legs and genital segment are described in detail with slight variations from the original description.

5. CONCLUSION

Elasmobranchs (sharks, skates and rays) are very important in commercial fisheries in various parts of the world including India. Most are excellent as human food. This is the first examination of marine fish parasitology from the point of view of the host fishes from the Nellore coast Bay of Bengal, India. In this study the whole host fish was examined for ectoparasites and a detailed study was accomplished with SEM studies. Scanning electron microscopic studies enable description of additional morphological details of parasites. The present study recorded *R. acutus* as a host for *K. minuta* in new locality (Nellore coast) from the east coast of India. Acquired knowledge of the whole parasite assemblage may also serve as an indicator to study the host shark ecology. This means that the parasites while intrinsically interesting and important ecologically, can also be used as a tool to further understand the biology and ecology of the host species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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