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Research Note

Redescription and phylogenetic analyses of *Thaparocleidus gomtius* and *T. sudhakari* (Monogenea: Dactylogyridae) from *Wallago attu* (Siluriformes: Siluridae) in India

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Summary

Two species of *Thaparocleidus* Jain (1952a) were found harboring *W. attu* from the Ganga River at two localities, Meerut and Farrukhabad, Uttar Pradesh, India, during the period of 2013 – 2015. Morphology and morphometric study of specimens identified as *Thaparocleidus gomtius* (Jain, 1952a) Lim, 1996 and *T. sudhakari* (Gusev, 1976) Lim, 1996. Molecular analyses using the 18S rRNA gene confirmed the validity of *T. gomtius* and *T. sudhakari* and demonstrated that both the species clustered with other *Thaparocleidus* species from different geographical regions. We aim at reassessing the taxonomy and establishing the phylogenetic relationships among these two redescribed species with other representatives of the genus *Thaparocleidus*.
Keywords: Monogenea; *Wallago attu; Thaparocleidus;* morphology; molecular analysis; 18S rDNA; India

Introduction

Siluriforms fish are amongst the most dominant species and playing an important role in the freshwater ecosystem of South-Central Asia. These fish have considerable commercial importance, mainly used for food or farmed. *Wallago attu* (Bloch and Schn., 1801) is one of the most commercially exploit fish in India. Till now, studies on the monogenetic infection proved that this fish harboring many species that included the genera *Bychowskyella* Akhmerow 1952; *Thaparocleidus* Jain, 1952b; *Neocalceostoma* Tripathi, 1959; *Cosmetocleithrum* Kritsky *et al.* 1986; *Hamatopeduncularia* Yamaguti, 1963 and *Rhamnocercus* Monaco *et al.* 1954. In all the genera harboring *W. attu*, *Thaparocleidus* Jain, 1952b is the most dominant and species rich genus. Sixteen nominal species of *Thaparocleidus* have been described from *W. attu* in India till now and many of them are considered as *species inquirendae* by Lim et al., 2001 as their descriptions were inadequate.

The examination of monogenean from gill filaments of *W. attu* from Ganga River in India revealed the presence of two species of *Thaparocleidus i.e., T. gomtius* (Jain, 1952) Lim, 1996 and *T. sudhakari* (Gusev, 1976) Lim, 1996. Although, both species were described earlier but was relied only on morphological studies. The previous studies lacking in describing detailed morphometrices and molecular systematic analysis which encouraged for a redescription to confirm and validate them. Presumed similarities in the male copulatory organ of *T. gomtius* and *T. sudhakari* provoked a comparative approach to establish a clear cut difference between them, which was further supported by molecular phylogenetic analysis. As far as the host fish *W. attu* is concerned, only six sequences of monogenean species (basically of 28S ribosomal DNA) belonging to the genus *Thaparocleidus* from India are available in GenBank. There is scarcity in availability of 18S rDNA sequence for any species of *Thaparocleidus* parasitizing *W. attu* from India for comparing and elucidating correct phylogenetic status of concerned species. The result of the assessment of phylogenetic relationships within the monogeneans, inferred from

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18S rDNA gene mainly of species of the family Dactylogyridae is presented herein.

The irrelevant information, inappropriate identification, no relation establishment regarding the generic or specific level in monogenean group, frequent synonymizing of various doubtful species and many more issues caused by morphologies from India have resulted in a shift to the use of molecular data. The present work employed the use of 18S rDNA molecular data which is a novelty in the resdescription of *T. gomtius* and *T. sudhakari*.

Materials and Methods

A total of 52 and 20 host fish of Ganga River were brought from fish markets or directly from fishermen from Meerut (29° 01' N, 77° 45' E) and Farrukhabad, (27° 24' N 79° 37' E) respectively of state Uttar Pradesh in India during 2013 – 2015. Fish specimens were kept in aerated jar and brought to the laboratory for helminthological examination. Froese and Pauly (2001) method was employed to confirm the specific identity of host fish. External examination of the host was done to observe any signs of abnormalities such as lesions, bruising or deformities. For the collection of monogenean worms, gill arches were removed, placed in petri dish contained saline water and worms were detached from gills using a strong current of water. The detached monogeneans were picked up using a fine needle under a dissecting microscope (Motic SMZ-168 series) and then transferred individually into a drop of glycerine on a slide for the preparation of semi-permanent slides. The preparation was then covered with a cover slip and sealed using a sealant for examination of sclerotized structures. Monogeneans were stained with acetocarmine, dehydrated through ascending grades of alcohol and mounted in Canada balsam for preparation of permanent slides. From these preparations, drawings were made with Camera Lucida connected to Motic digital microscope (DMB series). All measurements were made directly from drawings. All measurements are in millimeter unless otherwise stated.

For species identification and description, there are different types of measurements required. Measurements of haptor armaments and male copulatory organ (MCO) employed in the present study were adopted from the relevant published account of Gusev (1973) for Dactylogyridae and different morphotypes were used to characterize anchors, bars and hooks (Gusev, 1985).

After the collection of parasites from the gill filaments, each worm was observed under Motic Microscope and ascertained the specific identification, then preserved in 95 % ethanol at -20 °C for molecular analysis. DNA was extracted using a Qiagen DNeasy™ tissue kit (Qiagen, Germany) according to the manufacturer's instructions and stored at -20 °C until further processing. The partial fragment of 18S rDNA was amplified using the following primers, Worm A: GCGAATGGCTCATTAAATCAG; 1270R: CCGTCAATTCCTTTAAGT; 930F: GCATGGAATAATGGAATAGG; Worm B: CTTGTTACGACTTTTACTTCC (Littlewood and Olson 2001) and S1: ATTCCGATAACGAACGAGACT; IR8: GCTAGCTGCGTTCTTCATCGA (Šimková *et al*., 2003). Each amplification reaction of 25 µl contained 3 µl template DNA, 2.5 µl 10X PCR buffer (Biotools, Spain), 3.4 µl dNTPs (deoxynucleotide triphospates), 0.9 µl of each primer, 1 µl taq polymerase (1U; Biotools, Spain) and 13.3 µl water. PCR cycle was carried out with the following steps: 3 min at 94 °C with an initial denaturation followed by 40 cycles for 40 sec at 94 °C for further denaturation, annealing for 45 sec at 55 °C for all primer pairs, 1 min at 72 °C followed by a final extension for 10 min at 72 °C. An aliquot (4 µl) of each amplicon was checked on 1 % Tris-Acetate-EDTA buffer gel, stained with ethidium bromide and visualized under ultraviolet light. For sequencing, PCR products were directly sequenced using a Big Dye Terminator vr. 3.1 cycle sequencing kit in ABI 3130 Genetic Analyzer, Applied Biosystems (Foster City, USA) as recommended by the manufacturer with the above primers.

Amplified forward and reverse sequences of the 18S rDNA gene were assembled with Bioedit (Hall, 1999). CLUSTAL W (Thompson *et al*., 1994) was used for multiple alignments with the related sequences based on nucleotide similarities from GenBank using BLAST search. Nucleotide distance matrices were calculated using the *p*-distance model in MEGA vr. 6 (Tamura *et al*., 2013). Phylogenetic analyses of the nucleotide sequences were performed using Maximum Likelihood (ML) and Bayesian inference (BI). Firstly, the dataset was tested in the nucleotide substitution model of best fit using ModelTest vr. 3.07 (Posada & Crandall, 1998) and the best-fitting model was chosen using the Akaike Information Criterion (AIC) and the model GTR + G + I was selected. The phylogenetic reconstruction inferred by Maximum Likelihood (ML) using MEGA with bootstrap analysis based on 1000 replicates. Bayesian inference (BI) was performed using Topali 2.5 (Milne *et al.,* 2009), substitution model was tested by the Bayesian Information Criterion and GTR + I + G was chosen. BI analysis was run for 1,000,000 generations, sampling every 100th tree and discarding 'burn in' first 25 % of the sampled tree. Molecular phylogenetic analysis of 18S rDNA sequences obtained were performed with the closely related sequences of family Dactylogyridae retrieved from GenBank, especially *Thaparocleidus* in order to get the correct taxonomic status of concerned species. The 18S rDNA sequence of *Quadriacanthus* sp. (HG491496) was taken as outgroup.

Results and Discussion

Thaparocleidus gomtius (Jain, 1952a) Lim, 1996 (Fig. 1 and 2)

Redescription: Body elongated with narrow anterior and broad posterior end, divided into cephalic, trunk, peduncle and haptor region. Cephalic region contains four pairs of head organs and two pairs of eye spots, anterior and posterior, latter being larger in size. Followed by eyespot is muscular pharynx. Intestine bifurcated, crura confluent posteriorly, anterior to haptor. Vitellaria densely scattered over intestinal caeca. Gonads situated inter-caecal. Testis elongated, post-ovarian, post-equatorial. A fine tube, vas deferens

Table 1. Morphometrics (in mm) of *T. gomtius* (Jain, 1952 a) Lim, 1996 and *T. sudhakari* Gussev, 1973.

***Thaparocleidus* sps.** **Body features**	***T. gomtius*** **(Jain, 1952a) Lim, 1996**	***T.gomtius*** **Present observation**	***T. sudhakari*** **Gussev, 1973**	***T. sudhakari*** **Present observation**
Body length	0.9 – 0.96	0.903 (0.710 – 1.100)	0.50	0.561 (0.540 – 0.570)
Body width	0.075	0.162 (0.155 – 0.171)	0.15	0.162 (0.160 – 0.164)
Pharynx length	0.04	0.050 (0.040 – 0.063)	–	0.019 (0.018 – 0.021
Pharynx width	0.045	0.042 (0.035 – 0.051)	–	0.019 (0.018 – 0.020)
Haptoral length	0.11	0.088 (0.062 – 0.115)	–	0.096 (0.095 – 0.098)
Haptoral width	0.09	0.118 (0.092 – 0.144)	–	0.081 (0.079 – 0.081)
Total length of dorsal anchor	0.075 – 0.085	0.077 (0.074 – 0.081)	0.046 – 0.050	0.048 (0.046 – 0.052)
Main part length of dorsal anchor	–	0.067 (0.064 – 0.069)	0.010 – 0.013	0.039 (0.038 – 0.041)
Inner root length of dorsal anchor	–	0.017 (0.016 – 0.017)	–	0.008 (0.007 – 0.010)
Outer root length of dorsal anchor	–	0.002 (0.001 – 0.002)	–	0.001
Recurved point of dorsal anchor	–	0.038 (0.035 – 0.040)	0.025 – 0.028	0.026 (0.025 – 0.027)
Dorsal anchor patch length	0.025 – 0.03	0.025 (0.024 – 0.028)	0.010 – 0.015	0.012 (0.011 – 0.015)
Dorsal anchor patch width	–	0.009	0.005 – 0.006	0.005 (0.004 – 0.005)
Dorsal bar length	0.03 – 0.04	0.045 (0.040 – 0.049)	0.020 – 0.028	0.030 (0.029 – 0.031)
Dorsal bar width	–	0.007 (0.006 – 0.007)	0.003 – 0.006	0.005 0.004 – 0.007
Total length of ventral anchor	0.028 – 0.032	0.031 (0.027 – 0.035)	0.021 – 0.024	0.024 (0.023 – 0.027)
Main part length of ventral anchor	–	0.022 (0.021 – 0.023)	0.018 – 0.020	0.021 (0.019 – 0.024)
Inner root length of ventral anchor	–	0.007	0.003 – 0.005	0.006 (0.006 – 0.007)
Outer root length of ventral anchor	–	0.004 (0.003 – 0.004)	0.002 – 0.004	0.004 (0.004 – 0.006)
Recurved point of ventral anchor	–	0.019 (0.016 – 0.020)	0.013 – 0.015	0.015 (0.014 – 0.017
Ventral bar length	0.06 – 0.7	0.029 (0.028 – 0.030)	–	0.020 (0.019 – 0.020)
Ventral bar width	–	0.002	–	0.003
Hook length	0.01 – 0.012	0.019 (0.019 – 0.020)	0.021 – 0.014	0.016 (0.015 – 0.017)
Copulatory tube length	0.03 – 0.032	0.030 (0.030 – 0.033)	Straight; 0.040 – 0.058	Straight; 0.045 (0.042 – 0.047)
Accessory piece length	0.046 – 0.05	0.049 (0.049 – 0.050)	0.030 – 0.040	0.034 (0.034 – 0.035)
Accessory piece horse – shoe part	–	absent	–	absent
Vaginal length	–	not found	–	not found
Testis length	–	0.116 (0.115 – 0.118)	–	0.165 (0.165 – 0.172)
Testis width	–	0.076 (0.071 – 0.077)	–	0.124 (0.121 – 0.127)
Ovary length	–	0.151 (0.149 – 0.154)	–	0.177 (0.175 – 0.179)
Ovary width	–	0.081 (0.079 – 0.082)	–	0.124 (0.119 – 0.129)
Egg length	–	0.084 (0.082 – 0.084)	–	0.087 (0.084 – 0.091)
Egg width	–	0.075 (0.074 – 0.076)	–	0.059 (0.053 – 0.061)

arises at anterior end of testis, goes up to anterior of body, sometimes forming loop at left intestinal caeca and long finger like blind seminal vesicle that opens at the base of cirrus tube by a small ejaculating duct. MCO made up of a cirrus tube and an accessory piece. Cirrus tube double walled throughout its length, having a maximum width at its distal end measures 0.004. Accessory piece foliate type, with bifurcated end. Two very well developed prostatic reservoir of size 0.055 (0.052 – 0.057), open at the base of cirrus. Ovary oval to elongated, post-equatorial, pre-testicular. Ova large and nucleated. Egg spherical, double walled with spur.

Opisthohaptor distinctly set off from the body proper by a short peduncle which is devoid of vitelline follicles. Each anchor is moderately stout, roots slightly diverging, shaft more or less straight and point deeply recurved and pointed. Dorsal anchor with strong recurved point, elongated inner root but almost inconspicuous outer root. Shaft cylindrical, strengthened by sleeve sclerites. At the

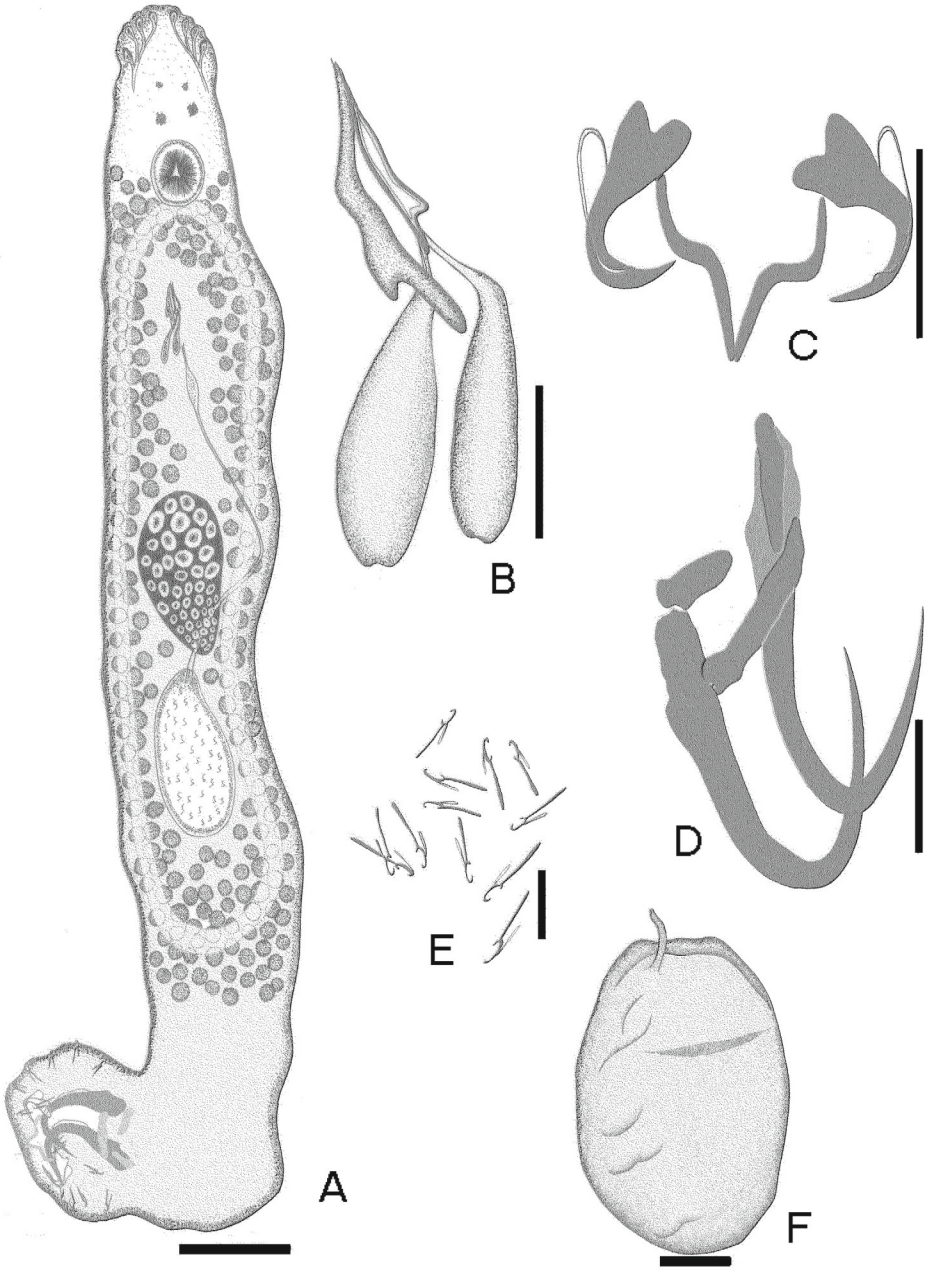

Fig. 1. *Thaparocleidus gomtius* (Jain, 1952a) Lim, 1996. (A) Whole mount. (B) Copulatory complex with prostate glands. (C) Ventral anchor and ventral bar. (D) Dorsal anchors with patches and dorsal bar. (E) Hooks. (F) Egg. Scale bars A), 0.1 mm, B), C), D) 0.025 mm, E), F) 0.012 mm.

base of inner root of dorsal anchor small inwardly directed conical supporting patches present. Dorsal transverse bar type with upwardly projecting ends. Ventral anchors sharply recurved almost straight point, small bifid roots, outer root smaller than inner one, shaft cylindrical and stout, strengthened by sleeve sclerites. Ventral bar V-like, curved. Marginal hooks seven pairs, composed of a sickle 0.0063 (0.060 – 0.067) and a handle 0.011 (0.011 – 0.012). Sickle consists of proximal and distal parts. Handle attached to the ventral part of sickle. Sickle-filament loop is a fine tendon like structure, attached at basal part of sickle on its inner root. An articulating portion of handle is slender and straight. Heel slightly swollen for provide the site of attachment to muscles. All the body measurements are given in Table 1.

Remarks: Since the original description by Jain (1952a) as *Haplocleidus gomtius* from *W. attu* in India, this species has been transferred to *Thaparocleidus* by Lim (1996). Pandey *et al.* (2003)

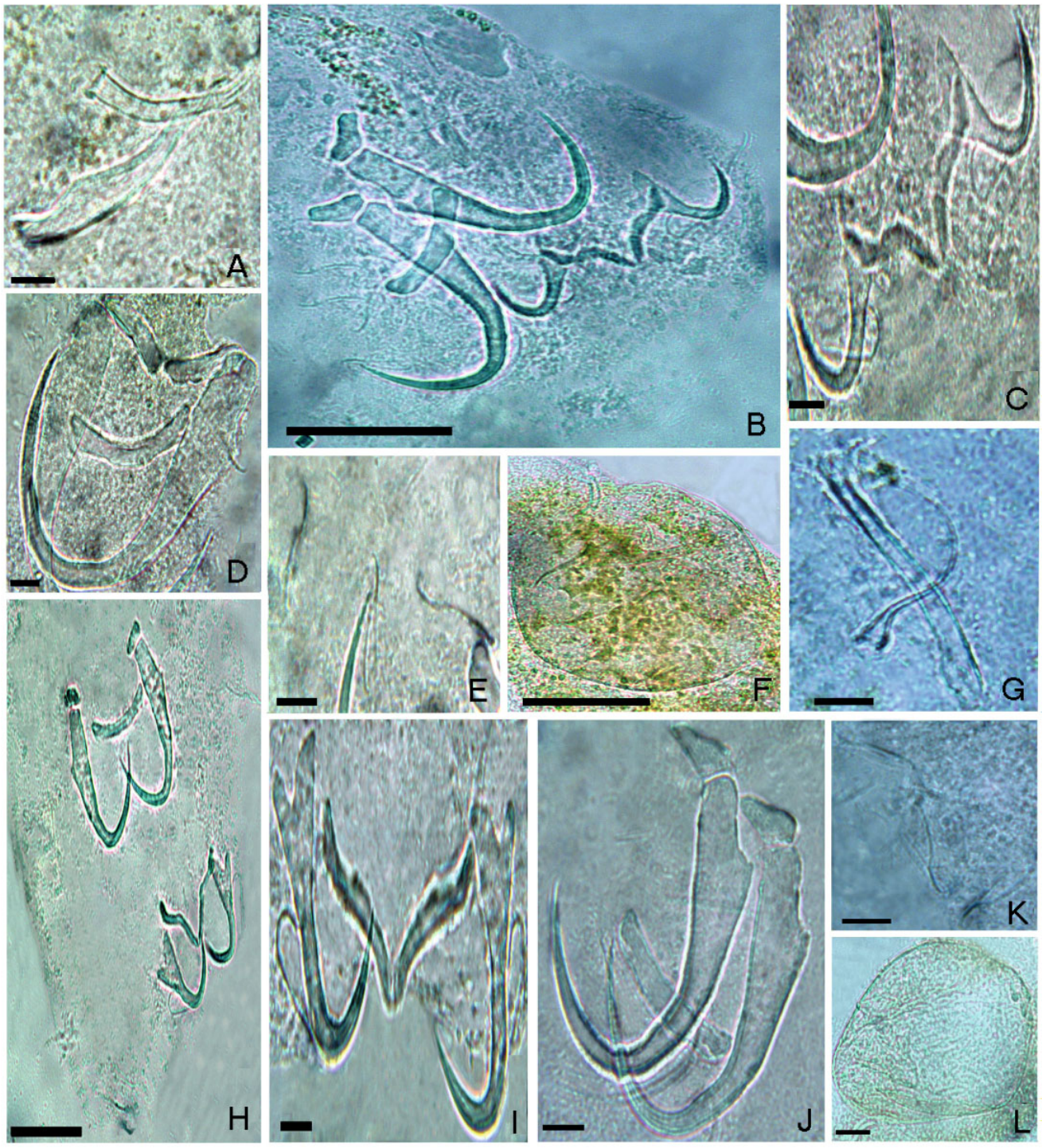

Fig. 2. Microphotographs of *T. gomtius* (A – F) and *T. sudhakari* (G – L). (A) Copulatory complex. (B) Haptoral armature. (C) Ventral anchor and ventral bar. (D) Dorsal anchors with patches and dorsal bar. (E) Hooks. (F) Egg. (G) Copulatory organ. (H) Haptoral armature. (I) Ventral anchor and ventral bar. (J) Dorsal anchors with patches and dorsal bar. (K) Hooks. (L) Egg. Scale bars A), C – E), G), I – K), 0.01 mm, B), F), H) 0.05 mm, L) 0.02 mm.

redescribed the same species and observed some more detailed morphological structures like 7 pairs of marginal hooks, vas deferens looping around left intestinal caeca and forming seminal vesicle that were deficient in the original description by Jain (1952b) also observed in the present study. The present species is different from *T. wallagonius* Jain (1952b) as the cirrus tube and vaginal tube are not coiled. It differs from *T. indicus* since the accessory piece is found to forked at the proximal end and the outer root of ventral anchor is longer than the inner root. It also differs from *T. sudhakari* (Gusev, 1976) Lim, 1996 in the morphology of MCO. The present species is characterized by having simple stick-like accessory piece with the forked proximal end, winged ventral anchor having an inner and outer root of almost similar length, broad stick shaped dorsal bar, dorsal anchor having stumpy outer root and characteristic dorsal patches, a pair of prostate glands present near the opening of the male genital pore, spherical double walled egg with a spur.

Host: *Wallago attu* (Bloch and Schn., 1801) (Siluriformes: Siluridae); *Locality:* Meerut (29° 01' N, 77° 45' E) and Farrukhabad, (27° 24' N 79° 37' E), Uttar Pradesh, India; *Site of infection:* Gill filaments; *Specimens studied*: Thirty-two; *Material deposited*: Prepared slides were deposited in the Museum d'Histoire Naturelle, Geneva, Switzerland (MHNG-INVE-91845) and the Museum, Department of Zoology, Chaudhary Charan Singh University, U.P., India (HS/monogenea/2015/11); *Representative sequence deposited*: The 18S rDNA sequence of *T. gomtius* was deposited in GenBank under the accession number KX462989 (815 bp); *Synonyms: Haplocleidus gomtius* Jain (1952a); *Silurodiscoides gomtius* (Jain, 1952) Gusev (1973); *Parancylodicoides gomtius* (Jain, 1952) Dubey *et al.* (1992).

Molecular data: The 18S rDNA sequence of *T. gomtius* is most closely related to Indian gill-infecting *Thaparocleidus* species, *T. sudhakari* (94.9 – 95 %) and clustered together with the same species in phylogram (Fig. 3). ML and BI analyses of the sequences

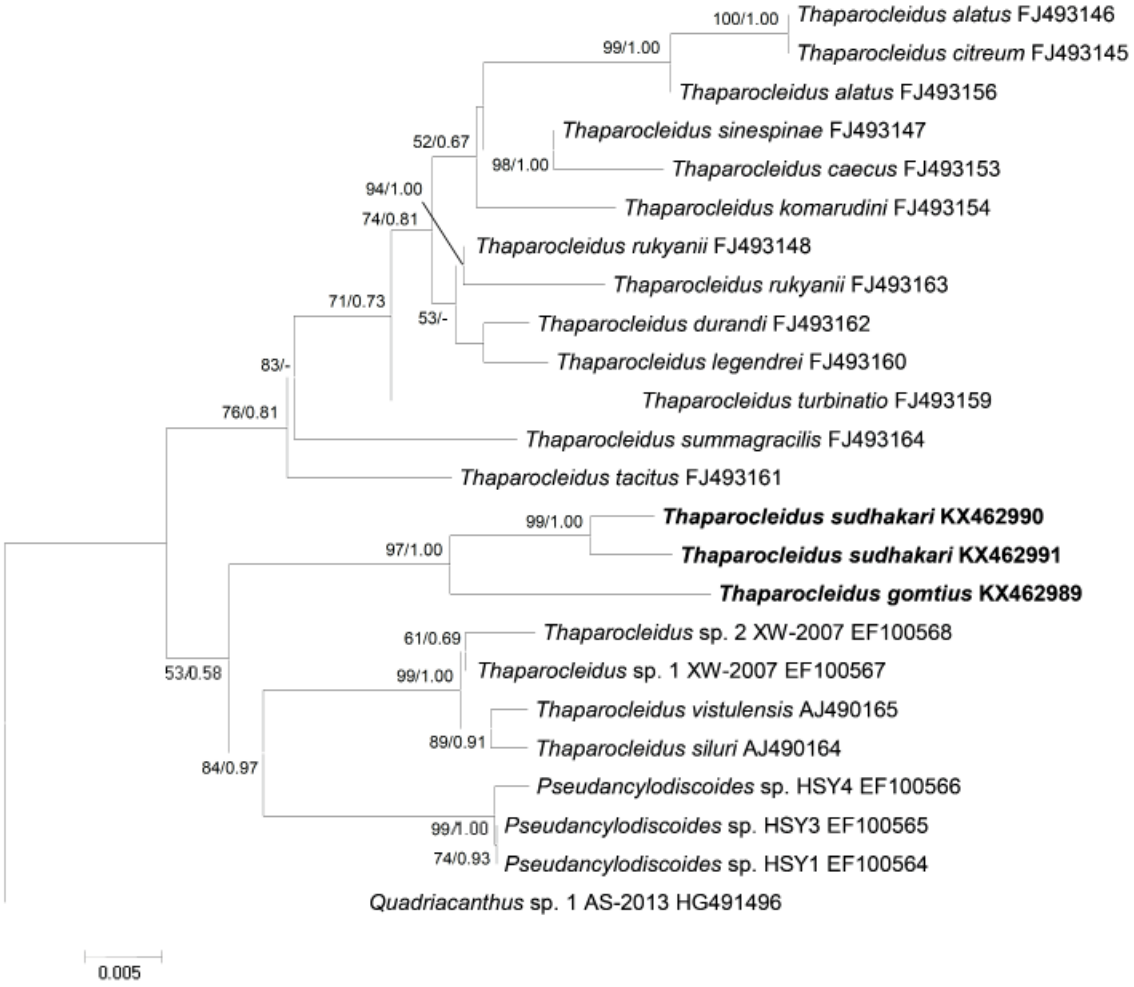

Fig. 3. A phylogenetic reconstruction based on the 18S rDNA sequence demonstrating the positions of *Thaparocleidus gomtius* and *Thaparocleidus sudhakari* with other monogenean species. The tree was generated by maximum likelihood (ML) method. Numbers at nodes indicate bootstrap values (1000 replications) ML and posterior probabilities (BI) respectively. Unsupported nodes by BI are marked with a hyphen. GenBank accession numbers are listed along the species names. Species examined in this study are indicated in bold.

generated highly similar topologies. The analysed sample differed from all of the available reference sequences in the GenBank database. The close similarities of 18S sequence of *T. gomtius* from India was also found with *Thaparocleidus* sp. 1 (EF100567) and *Thaparocleidus* sp. 2 (EF100568) from China and *T. vistulensis* (AJ490165) and *T. siluri* (AJ490164) from Czech Republic.

Thaparocleidus sudhakari (Gusev, 1976) Lim, 1996 (Fig. 2 and 4)

Redescription: Body elongated, bluntly pointed at anterior end, length varies from small to moderate size. Prohaptor equipped with four pairs of head organs and two pairs of compact eyespot, posterior pair being slightly larger than the anterior one. Pharynx round and muscular. Intestine simple, bifurcated and crura confluent posteriorly. Testis single, elongated oval, inter-caecal, post-equatorial. Half of testis overlapped by ovary. From anterior end of testis, a fine tube, called vasa defferentia arises makes a loop around right intestinal caeca and dilated to form a fusiform seminal vesicle for storage purpose, at level of vagina on opposite side. Seminal vesicle opens at base of MCO. MCO simple, Initial-type, consists of a cirrus tube and an accessory piece. Cirrus tube having widened proximal part and strongly curved distal part. Accessory piece is in form of double walled chitinoid structure, long curved gutter shaped, almost equal in width throughout length, connected with initial part of cirrus and finished by extension. Ovary elongated, oval, inter-caecal, post-equatorial, posteriorly overlaps to testis. Vaginal armament present at slightly left to middle part of the body i.e., sinistral in position, has a shape of a cylindrical tube, tilt at middle portion and expanded to open to outside. Egg spherical, double walled without any spur.

Opisthohaptor very well demarcated from body proper by short peduncle. Dorsal anchor with a well defined base, main part narrows into strongly recurved points, long inner root and stumpy outer root, shaft more or less cylindrical and equipped with sleeve sclerites. Dorsal patch widened at one termination and narrowed towards another. Dorsal transverse bar with weakly or without widening in its medial part. Ventral anchors strong recurved point, broad base. Ventral bar paired, V- shaped. Marginal hooks larval-type, 7 pairs, all similar, comprises of a protruding heel of sickle, a long handle and a sickle filament loop. All the body measurements are given in Table 1.

Remarks: The present species has been first described by Gusev (1973) from *W. attu* in India. He proposed that *T. sudhakari* showed close resemblance with *T. infundibulovagina* (Yamaguti, 1942). Later Lim (1996) redescribed it and synonymies it as *T. indicus* (Kulkarni, 1969) Lim, 1996. Though both the species show characteristic differences in the morphology of their MCO and haptoral armature, therefore the present observation disapproved this synonymization. There is a significant difference in the size of outer root of ventral anchors, shape of dorsal bar and in the MCO. Cirrus tube of *T. indicus* is slightly wavy at its distal end, but straight in the rest part whereas in *T. sudhakari*, the cirrus tube is found to be strongly curved in all the studied specimens and the tube ends at the mid length of accessory piece. *T. sudhakari* differs significantly from *T. wallagonius* Jain, 1952b in having coiled cirrus tube. The present species is also different from *T. gomtius* (Jain, 1952a) Lim, 1996 where the accessory piece is forked at its proximal end. Therefore, *T. sudhakari* can be distinguished from its congener species by its characteristic features like dorsal bar stick shaped with an almost equal width throughout its length, two piece ventral bar, each are of S-shaped, cirrus tube curved and knobbed at its proximal end, accessory piece long, plate like and 7 pairs larval type marginal hooks. The morphometric data of *T. sudhakari* from the present study falls within the ranges of Gusev (1973).

Host: Wallago attu (Bloch and Schn., 1801) (Siluriformes: Siluridae); *Locality:* Meerut (29° 01' N, 77° 45' E) and Farrukhabad, (27° 24' N 79° 37' E), Uttar Pradesh, India; *Site of infection:* Gill filaments; *Specimens studied:* Forty-five; *Material deposited:* The slides were deposited in the Museum d' Histoire Naturelle, Geneva, Switzerland (MHNG-INVE-91844) and the Museum, Department of Zoology, Chaudhary Charan Singh University, U.P. (HS/monogenea/2015/13); *Representative Sequence*: The 18S rDNA sequence of *T. sudhakari* was deposited in GenBank under the accession numbers KX462990 (1310 bp) and KX462991 (1290 bp); *Synonyms: Silurodiscoides sudhakari* Gusev (1976); *Paracylodiscoides sudhakari* (Gusev, 1976) Dubey *et al*., 1992.

Molecular data: Two parallel samples of *T. sudhakari* isolate 1 and isolate 2 were identical regarding their 18S rDNA sequences (100 %). A BLAST search presented no identical was found with any other *Thaparocleidus* species represented in GenBank. The most similar 18S rDNA sequences in GenBank to *T. gomtius* extracted from the same host from India (Fig. 3). Maximum likelihood and Bayesian inference both placed *T. sudhakari* as a putative sister taxa to *T. gomtius*, both species clustered together and formed a monophyletic clade. Both maximum likelihood and Bayesian inference analyses gave highly similar tree topology of the position of *T. sudhakari* (Fig. 3).

The two different forms of monogenean collected from *W. attu* in India shares somewhat similar morphologies in their haptoral armature. There are minor differences in the size and shape of dorsal anchor, dorsal bar and ventral anchor which could not be neglected without further study. The size and shape of the male copulatory organ are remarkably different in both forms. All these differences and similarities in the sclerotized structures prompt us to confirm the result with the addition of all minute morphometric study and DNA sequence analyses. It is also noted that most of the Indian monogenean species are considered as species inquirendae due to poor description and unavailability of data to analyze further. All these reasons necessitate to redescribed the monogenean species for its accurate identification and validation. Twenty genera of monogeneans harboring siluriform fish, *W. attu*, had been reported till date. With the span of time, there have lots of work being done on the correct evaluation of all these genera. Up to now, assessment of these 20 dactylogyridean genera indi-

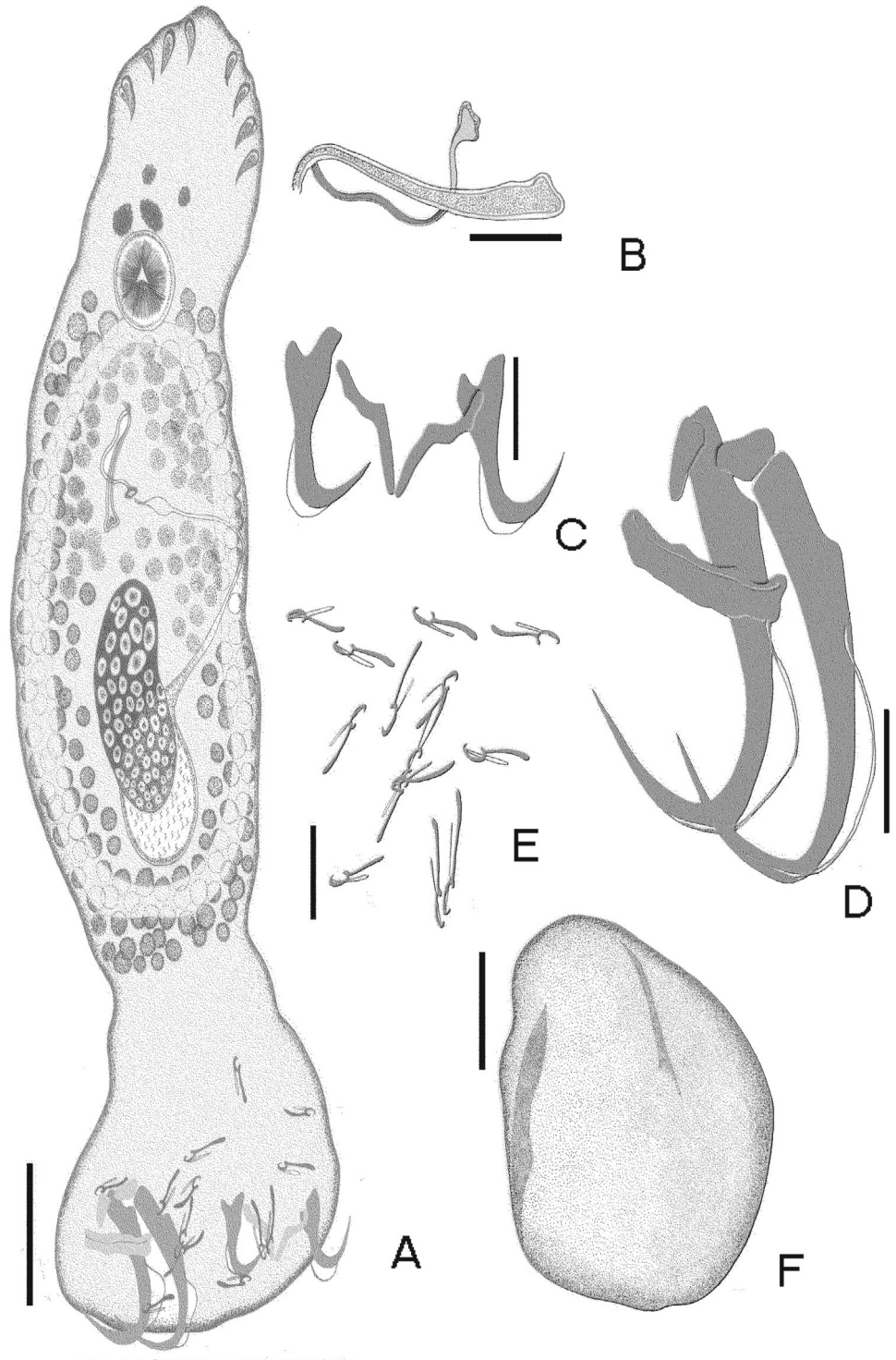

Fig. 4. *Thaparocleidus sudhakari* (Gusev, 1976) Lim, 1996. (A) Whole mount. (B) Copulatory complex. (C) Ventral anchor and ventral bar. (D) Dorsal anchor with dorsal patch and dorsal bar (E) Hooks. (F) Egg. Scale bars A), 0.05 mm, B), C), D), E), F) 0.012 mm.

cates that only 7 are valid, while the other monogenean genera are listed as synonymous (Lim and Lerssutthichawal, 1996; Lim, 1998; Lim *et al.,* 2001). It is very well known that molecular data is used to establish differentiation within species that showing a high degree of morphological similarities. It can also be assumed that molecular biological studies could be used in making an assessment whether the morphologically similar species are the same or different species (Šimková *et al.* 2013). DNA sequence studies help in differentiating the two species having very high levels of morphological similarities. DNA sequences from dactylogyridean monogeneans obtained in the duration of this study and DNA sequences deposited in the GenBank were analyzed to figure out relationships of studied monogeneans with reference sequences belong to Dactylogyridae.

Two forms of monogeneans collected were identified as *T. gomtius* and *T. sudhakari* following the original description of Jain (1952b), Gusev (1973) respectively. The prevalence of *T. gomtius* and *T. sudhakari* were found to be 77.7 % and 91.6 % whereas mean infection intensity was 12.6 and 19.9 respectively. When comparing morphological and morphometric characteristics, *T. sudhakari* although shares similarities with *T. gomtius* in the shape of their haptoral armature (including dorsal anchor, ventral anchor and ventral bar) but possessing remarkably different male copulatory organ. As compared to the total length of ventral anchor, the inner root of *T. sudhakari* is longer than outer root whereas in *T. gomtius* the inner and outer root are of almost similar in length. The cirrus tube is curved with a widened initial part in *T. sudhakari* whereas it is knobbed at its proximal end in *T. gomtius*.

In the phylogram constructed from 18S rDNA, *Thaparocleidus* sp. 1 (EF100567), *Thaparocleidus* sp. 2 (EF100568), *T. vistulensis* (AJ490165) and *T. siluri* (AJ490164) converged in a cluster, which is apparently different from the cluster formed by Indian *Thaparocleidus* spp. Further members of *Thaparocleidus* from China and Czech Republic were sister to the clade formed by *T. gomtius* and *T. sudhakari* from India. This paper demonstrated the position of *T. sudhakari* and *T. gomtius* within other members of Dactylogyridae. These two species were not marked earlier with the molecular marker reported here. We have selected closely related sequence of mostly members of *Thaparocleidus* species based on BLAST search. Phylogenetic tree constructed by 18S rDNA shows the monophyly of *T. gomtius* and *T. sudhakari* and the result also predicts paraphyly of the representatives of *Thaparocleidus* from different geographical regions, forming three different clades branched at different nodes. Species of Indian *Thaparocleidus* spp. forming a separate clade with other representatives of the same genus from different geographical region nested inside of it. Thirteen representatives of *Thaparocleidus* forms a separate monophyletic clade which is far distinct from the clades stated earlier. The specimens of *T. sudhakari* sequence in the present study show close relationship and putative sister species with *T. gomtius* sequenced herein.

The current study showed that the combined application of morphological and molecular methods is useful in the correct description and validation of erroneously or poorly described monogenean species and it significantly improve the understanding of both genetic and morphological variability of species level.

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