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Research Article

Molecular characterization of Indian species of the genus *Cornudiscoides* Kulkarni, 1969 (Monogenoidea: Dactylogyridae)

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Abstract
Molecular characterization and phylogenetic study based on partial sequences of 28S and 18S ribosomal DNA (rDNA) of sixteen Indian species of the genus *Cornudiscoides* (Monogenoidea: Dactylogyridae) were conducted to decode the genetic relationship between them and with other members of the family Dactylogyridae. Blastn searches disclosed the significant similarity among the species of the *Cornudiscoides* for large ribosomal subunits as well as for small ribosomal subunit showing genetic relatedness. The phylogenetic tree using neighbour-joining (NJ) and minimum evolution (ME) methods for 28S ribosomal subunit depicted that all *Cornudiscoides* species clustering in a single clade and forming sister clade with other members of the family Dactylogyridae and similar results were obtained from 18S ribosomal subunit. Thus, the present study demonstrated that both 28S and 18S ribosomal subunits are very helpful in discriminating *Cornudiscoides* species (intra or interspecific variation) and in the establishment of the evolutionary relationship among them.

Keywords: *Cornudiscoides,* Dactylogyridae, Large and small ribosomal subunit, Maximum likelihood methods, Neighbour-joining, Phylogenetic analysis

INTRODUCTION

Systematics is a branch of science that described the world's biodiversity and its interrelationships (Van Steenberge *et al*., 2015). Traditionally, taxonomy-based only morphological characters, but, given the constraints of morphology-based strategies in discriminating between cryptic species or decoding the variations of intraspecific variations, an integrative approach of taxonomy has been proposed (Dayrat *et al*., 2005). According to Schlick-Steiner *et al.* (2010) at least three impartial datasets: morphology, nuclear DNA and other supporting proof from another discipline should be used.
Hard parts, i.e. haptor and male and female copulatory complex, are the main diagnostic features in monogenoideans taxonomy. Changes in the environmental conditions like locality and geographic distribution of host and age of parasites, the hard parts may exhibit variation in their structure (Agrawal *et al*., 2020). Thus, morphology is alone not sufficient in identifying species. Eukaryotic genes encoding ribosomal RNA (rRNA) is not only used as an effective taxonomic tool but recognized as a potential target for species identification, differentiation and phylogenetic analysis of helminths parasites. The 28S and 18S ribosomal DNA (rDNA) fragments particularly lend themselves to study as they provide sequences along with constant sites that permit multiple alignments among or between homologues, and variable sites that give phylogenetic information (Hillis and Dixon, 1991). In the recent era of molecular taxonomy, the ribosomal RNA (typical nucleic acid) frequently targeted for sequencing in eukaryotes and prokaryotes both (Olsen and Woese, 1993).
Kulkarni (1969) erected the genus *Cornudiscoides* at Hyderabad and established three species *C. heterotylus* Kulkarni, 1969 (type species), *C. microtylus* Kulkarni, 1969 and *C. megalorchis* Kulkarni, 1969 from *Mystus tengara.* To date, 16 species of the genus *Cornudiscoides* have been described and are distributed throughout South East Asia (Agrawal *et al*., 2016). Earlier reports showed that large ribosomal subunit

(Mollaret *et al*., 1997 and 2000; Olson and Littlewood, 2002; and Verma *et al*., 2017) and small ribosomal (Matejusová *et al*., 2001, and Verma *et al*., 2017) useful to resolve relationships among monogenoidean parasites. In this study, twelve known species namely *C. heterotylus* Kulkarni, 1996; *C. mystusi* (Rizvi, 1971) Dubey *et al*., 1992; *C. proximus* Gusev, 1976; *C. geminus* Gusev, 1976; *C. agarwali* Agrawal and Vishwakarma, 1996; *C. bleekerai* Agrawal and Vishwakarma, 1996; *C. gussevi* Agrawal and Vishwakarma, 1996; *C. susanai* Agrawal and Vishwakarma, 1996; *C. tukarami* Agrawal and Vishwakarma, 1996; *C. sclerovaginalis* Devek and Pandey, 2007; *C. longicirrus* Agrawal *et al*., 2016; *C. aori* Agrawal *et al*., 2016 and four new species *C. tripathii* n. sp., *C. speratai* n. sp., *C. indicus* n. sp. and *C. falcatum* n. sp. were sequenced and partial sequences of the 28S and 18s ribosomal DNA were used to infer the relationships among the species of the genus *Cornudiscoides* and with the other members of the family Dactylogyridae.

MATERIALS AND METHODS

Collection of parasites

The hosts (commonly available freshwater food fishes for which ethical clearance is not required) were caught from River Gomati (Lucknow), Sai River (Lucknow), Manorama River (Basti), Betwa River (Jhansi), Amdhanpur Taal (Basti), Ramgarh Taal (Gorakhpur), and from the ponds of Mati (Barabanki) were examined Since January 2014 to January 2018. Collections were also made from the fish markets of Lucknow like Kaiserbagh, Daliganj, Dubbaga; Malihabad; Gonda, Sitapur, Hyderabad (Telangana) and Vizag (Andhra Pradesh). The live hosts were kept in plastic containers, aerated with battery operated aerators, brought to the laboratory, and maintained in glass aquaria. Fishes were identified with the help of Fish base (Froese and Pauly, 2014-2018), and Jayaram (1955). The gills of fishes were excised and were examined for the monogenoids. Living worms were studied under a phase-contrast microscope (Olympus CX41, Tokyo, Japan). The monogenoids were identified with the help of Pandey and Agrawal (2008). The gills infected with monogenoidean parasites were fixed in 3% formalin diluted with lukewarm water, temporary slides (glycerine mounts) and permanent slides ware prepared for morphometric study. The method for staining, mounting and illustration of parasites done according to Kristsky *et al.* (1986). For the molecular study, the gills were stored in 100% ethanol.

Molecular analysis

DNA (genomic) of the parasite (*Cornudiscoides*) was extracted from ethanol-preserved specimens using DNeasy Tissue Kit (Qiagen, Hilden, Germany) according to standard methods (Verma *et al*., 2018). A Partial region of 28S and 18s ribosomal DNA were amplified with the help of polymerase chain reaction (PCR) using the primers (Table 1) and reaction mixture prepared according to Verma *et al*., 2016 (Table 2). The thermal cycle started with 3 min at 94°C for initial denaturation; followed by 35 cycles of 30 s at 94°C, 30 s at 52°C for annealing, and 2min at 72°C (extension); and a final extension at 72°C for 10min followed by cooling at 4° C. PCR products were examined on 1% agarose gel, stained with ethidium bromide and visualized on a gel documentation system. Sequencing was carried out according to Verma *et al*. (2017).

Blastn (Basic Local Alignment Search Tool) was performed for partial sequences of both 28S and 18S rDNA to uncover the degree of resemblance between species of the genus *Cornudiscoide*s. ATGC calculator was used to evaluating the nucleotide composition among different species of the genus *Cornudiscoides* in this study. Sequences of the different species of the genus *Cornudiscoides* for 28S and 18S ribosomal subunit were amplified and sequenced and analysed with neighbour-joining and maximum likelihood methods. Sequences were reconstructed using Bioedit prior to analysis. Molecular evolutionary genetics analysis MEGA7 version 7.0 (Kumar *et al*., 2016), is software designed to infer phylogenetic relationship and pattern of evolution of nucleic acid and protein.

RESULTS AND DISCUSSION

Molecular characterization

The partial sequences of 28S and 18S rDNA genes were used to assess the genetic differentiation of sixteen species of Cornudiscoides and their phylogenetic relationship among other groups of monogenoidean parasites. The large and small ribosomal subunits are extremely useful to explain the phylogeny of monogenoideans at the level of family and subfamily (Šimková *et al*., 2006) and the nucleotide sequences of monogenoideans have sufficient phylogenetic information to decode the relationship among them (Cunningham *et al*., 2000).

Sixteen newly generated sequences ranged from 327-1041 base pair long for large ribosomal subunit and 483-1109 base pair long for small ribosomal subunit (Table 4, 5), were obtained. All the sequences are submitted to the GenBank, National centre for biotechnology information (NCBI) and their accession numbers are given in Table 3.

Blastn searches performed for 28S region depicted that most of the members showed the highest similarity with *C. agarwali* (Table 4) while Blastn search for 18S region, showed similarity with *C. susanai* (Table 5). The distribution of ATGC among different *Cornudiscoides* species deviated from species to species for both ribo-

Table 1. Primer pairs used for amplification of partial 28S and 18S ribosomal DNA and their sources.

Gene	Primer	Primer sequence	Sources
28S rDNA	Ancy55F (Forward)	GAGATTAGCCCATCACCGAAG (21 mer)	(Plaisance *et al*., 2005)
	LSU1200R (Reverse)	GCATAGTTCACCATCTTTCGG (21 mer)	(Plaisance *et al*., 2005)
28S rDNA	Universal primer (Forward)	ACCCGCTGAATTAAGCAT (18 mer)	(Shrivastava *et al*., 2013)
	Universal primer (Reverse)	CTCTTCAGACTTTTCAAC (18 mer)	(Shrivastava *et al*., 2013)
18S rDNA	Worm A (Forward)	ACGAATGGCTCATTAAATCAG (21 mer)	(Plaisance *et al*., 2005)
	Worm B (Reverse)	CTTGTTACGACTTTTACTTCC (21 mer)	(Plaisance *et al*., 2005)

Table 2. Concentration of Master Mix for partial 28S and 18S rDNA amplification.

Component	Volume (µl)	Final concentration
PCR buffer (10x)	3.0	1x
$MgCl_2$ (50mM)	0.9	1.5 mM
dNTP mixture (10 mM)	0.6	200 µM
Primer forward (10 µM)	1.2	0.4 µM
Primer reverse (10 µM)	1.2	0.4 µM
Taq DNA polymerase (5U/ µl)	0.4	2U/ µl
DNA	6.0	-
Milli Q water	16.7	-
Total volume	**30**	-

somal regions depicted in Table 4 and 5.

Phylogenetic analysis

Phylogenetic analysis was conducted among the members of the family Dactylogyridae for 28S and 18S ribosomal subunits. Newly obtained sequences along with twenty sequences belonging to different subfamilies members of the family Dactylogyridae, retrieved from GenBank, were used to evaluate their phylogenetic relationship. Evolutionary analyses were conducted in MEGA7 version 7.0 software (Kumar *et al*., 2016), using neighbour -Joining (NJ) and Minimum Evolution (ME) methods. Substitution, including transitions, transversions, gaps and missing data, were decimated. In the analyses, the codon positions (first, second and third) and non-coding sites were also included. The nodal values were estimated by bootstrapping (n=1000). Phylogenetic analysis based on these two methods produced identical tree topologies with the branch length 0.10 and 0.05 for 28S and 18S. The evolutionary distances were estimated with the help of the p-distance method.

However, the phylogenetic tree generated from both 28S and 18S rDNA region depicted that the members of the family Dactylogyridae are separated on the basis of subfamilies i.e. members of subfamily Ancylodiscoidinae, Ancyrocephalinae, Pseudodactylogyrinae and Dactylogyrinae. They clustered separately, forming sister clades, with each other and separate clade with a member of Sundanonchidae for 28S rDNA region (Fig. 1, 2). In the clade of Ancylodiscoidinae, all the species of *Cornudiscoides* clustered together and formed sister clade with *Thaparocleidus* spp. It is worth to note that, in the phylogenetic analysis of partial sequences of 28S and 18S ribosomal units, similar results were found, showing genetic relatedness among *Cornudiscoides* spp. while the separation of others reflects inter-specific/ intergeneric dissimilarities.

The species of the genus *Cornudiscoides* are chiefly differentiated by structure of their hard parts (haptor and genital armature) (Agrawal *et al*., 2020). The molecular study is conducted to complement morphometric analysis. Genetic distance portrays a degree of heterogeneity in the genetic constitution of taxa, therefore, becomes an ideal systematic tool (Fergusson, 2002). The ribosomal DNA of monogenoideans was used to evaluate phylogenetic relationship at the level of families and subfamilies (Plaisance *et al*., 2005; Šimková *et al*., 2003, 2006). In the identification of closely related parasites, PCR-based DNA sequencing technology provides an alternative approach (Nolan and Cribb 2005). Nucleotide sequences of monogenoideans have sufficient phylogenetic information to decode the relationship among them (Cunningham *et al*., 2000).

For the first time, the attempt has been taken for comprehensive analysis of sixteen species of *Cornudiscoides*, using 28S and 18S rDNA partial sequences to show the genetic relatedness among them. Different sets of primers amplified different range of 327 to 1041 base pairs for 28S region and 483 to 1109 base

Table 3. List of Monogenoideans and their accession numbers used into phylogenetic analysis.

S. No.	*Cornudiscoides* spp.	GenBank Accession Number	
		28S	**18S**
1	*C. heterotylus*	Submitted	MN187552
2	*C. mystusi*	KY091690	KY091691
3	*C. proximus*	KU358726	KU235550
4	*C. geminus*	KU235549 KU358727	KU358728
5	*C. agarwali*	KU208072 KU208071	KU928129
6	*C. bleekerai*	MK226180	MN176410
7	*C. susanai*	MK226179	MK396660
8	*C. gussevi*	-	MN219985
9	*C. tukarami*	MN176418	MN176391
10	*C. sclerovaginalis*	MK403005	-
11	*C. longicirrus*	KY009858	KY018927
12	*C. aori*	KY009859	KY018928
13	*C. tripathii* n. sp.	MK282177	MK402984
14	*C. speratai* n. sp.	MK575194	MK570612
15	*C. indicus* n. sp.	MN179357	-
16	*C. falcatum* n. sp.	MK418453	Submitted
17	*Thaparocleidus infundibulovagina*	EF100548	-
18	*Thaparocleidus indicus*	JX960419	-
19	*Thaparocleidus summagracilis*	-	FJ493164
20	*Thaparocleidus tacticus*	-	FJ493161
21	*Thaparocleidus durandi*	-	FJ493162.
22	*Euryhaliotrematoides berenguelae*	AY820615	-
23	*Euryhaliotrematoides microphallus*	AY820617	-
24	*Euryhaliotrematoides annulocirrus*	EU836195	-
25	*Euryhaliotrematoides* sp.1	-	EU836217
26	*Haliotrematoides spinatus*	-	JN054404
27	*Pseudodactylogyrus anguillae*	AJ969950	
28	*Pseudodactylogyrus* sp.	EF100540	
29	*Pseudodactylogyroides apogonis*	-	AB065115
30	*Pseudodactylogyrus haze*	-	AB065114
31	*Dactylogyrus anchoratus*	JX524546	-
32	*Dactylogyrus alatus*	MG792957	-
33	*Dactylogyrus falciformis*		FN391583
34	*Dactylogyridae* sp.4		EU836234
35	*Sundanonchus micropeltis*	AF218122	-
36	*Sundanonchus behuri*	GU830883	-

pairs for 18S region. The Blastn search disclosed the obscure relationship of these parasites with other species and showed the highest similarity with *Cornudiscoides* spp. for large as well as for small ribosomal subunit (Table 4 and 5).

The dispersion of ATGC bases (Table 4 and 5) for the two regions among *Cornudiscoides* spp. found deviated from species to species and exhibit clear-cut interspecific differences.

Among several sequences used in phylogenetic/cladistic analysis, it was found that rDNA is extremely useful in evidencing intra and inter-specific variations among parasitic species since they evolve with varying rates from extremely conserved (18S, 5.8S and 28S) to extremely variable (transcribed and non-transcribed or IGS) regions (Hillis and Dixon 1991). Comparative study of 28S and 18S regions of ribosomal DNA reveals considerable sequence similarity as well as differences to define relativeness and phylogenetic relationship. These subunits have a wide range of phylogenetic utili-

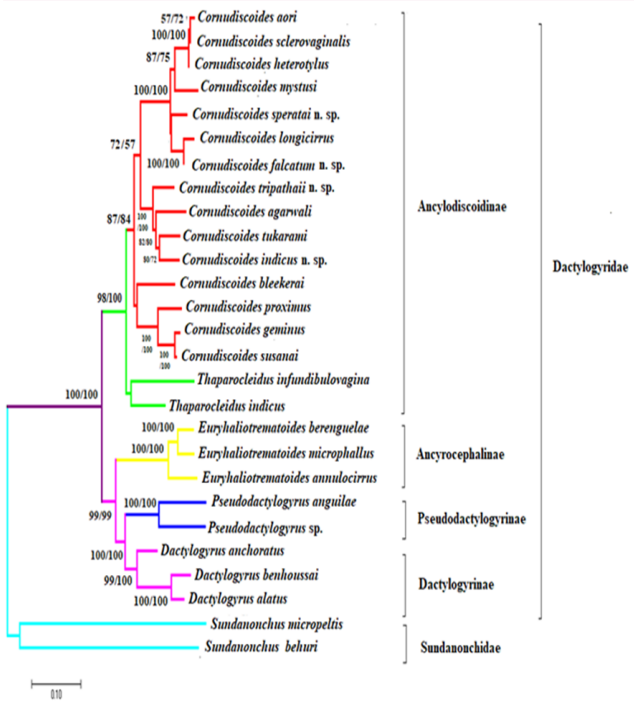

Fig.1. *Tree topology for rDNA for members of the family Dactylogyridae for 28S ribosomal subunit, using Neighbor* -Joining (NJ) and Minimum Evolution (ME) methods. *Bootstrap values support 1000 replicates below the nodes for NJ and above for ME.*

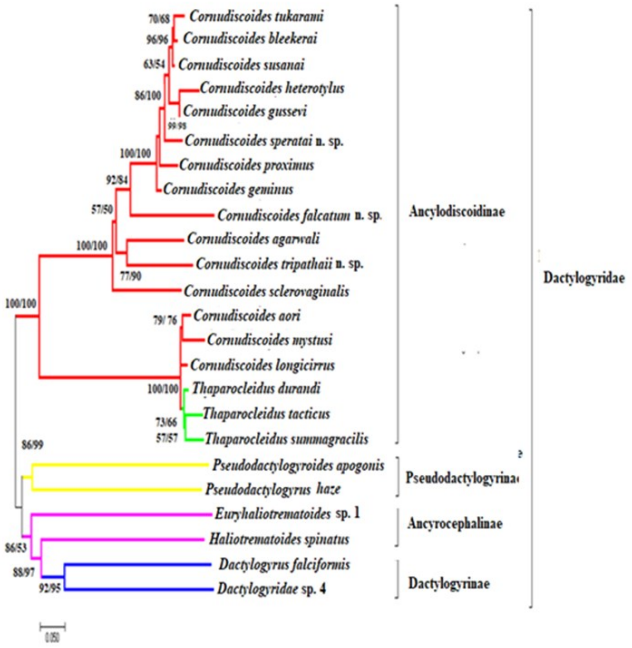

Fig. 2. *Tree topology for rDNA for members of the family Dactylogyridae for18S ribosomal subunits, using Neighbor* -Joining (NJ) and Minimum Evolution (ME) methods. *Bootstrap values support 1000 replicates below the nodes for NJ and above for ME.*

Table 4. Distribution of A, T, G, C contents % of GC and amplicon size along with blastn similarity of 28S rDNA of *Cornudiscoides* species.

***Cornudiscoides* spp.**	**Adenine (A)**	**Thymine (T)**	**Guanine (G)**	**Cytosine (C)**	**GC (%)**	**Amplicon size (bp)**	**Blastn similarity**
C. heterotylus	154	212	169	114	43.6	649	96.00% with *C. susanai*
C. mystusi	238	295	269	198	46.7	1000	92.24% with *C. aori*
C. proximus	220	274	236	156	44.2	886	95.30% with *C agarwali*
C. geminus	216	266	238	153	44.8	873	97.41% with *C. susanai*
C. agarwali	255	295	257	182	44.4	989	97.78% with *C. agarwali*
C. tukarami	269	297	283	192	46.6	1041	95.34% with *C. agarwali*
C. bleekerai	89	76	93	69	49.5	327	96.59% with *C. agawali*
C. susanai	190	239	185	126	42	740	96.00% with *C. agawali*
C. sclerovaginalis	170	226	187	125	44.1	708	98.72% with *C. aori*
C. longicirrus	285	262	279	209	46.9	1030	92.23% with *C. agarwali*
C. aori	246	294	265	183	45.3	988	99.72% with *C. sclerovaginals*
C. tripathii	178	228	206	147	46.5	759	98.05% with *C. geminus* 99.47% with *C.* species LW
C. speratai	161	219	177	122	44	679	92.92% with C. *longicirrus*
C. indicus	252	283	260	182	45.2	977	92.25% with *C. Proximus*
C. falcatum	209	279	240	175	46	903	98.92% with *C. longicirrus*

ty (high sequence similarity with the highly variable region) providing an easy alignment between taxa (Littlewood *et al*., 1998) and are useful in genetic characterization of species.

In the computed phylogenetic tree for 28S and 18S regions, the species of *Cornudiscoides*, formed a different clade with other subfamily members of the family Dactylogyridae. All *Cornudiscoides* species clustered together in a single clade, forming sister clade with *Thaparocleidus* spp. showing a close relationship

Table 5. Distribution of A, T, G, C contents % of GC and amplicon size of 18S rDNA of *Cornudiscoides* species**.**

***Cornudiscoides* spp.**	**Adenine (A)**	**Thymine (T)**	**Guanine (G)**	**Cytosine (C)**	**GC (%)**	**Amplicon size (bp)**	**Blastn similarity**
C. heterotylus	163	159	175	149	50.2	646	96.68% with *C. susanai*
C. mystusi	294	288	300	227	47.5	1109	98.24% with *C. longicirrus*
C. proximus	161	152	161	126	47.8	600	97.31% with *C. susanai*
C. geminus	132	122	125	104	47.4	483	98.17% with *C proximus*
C. agarwali	148	140	159	148	51.6	595	99.44% with *C. susanai*
C. tukarami	152	172	180	134	49.2	638	98.65% with *C. susanai*
C. gussevi	142	129	144	144	48-8	529	99.81% with *C. susanai*
C. bleekerai	181	204	211	164	49.3	760	98.79% with *C. susanai*
C. susanai	171	178	188	143	48	680	97.31% with *C. proximus*
C. sclerovaginalis	156	159	174	158	51.3	647	98.24% with *C. mystusi*
C. longicirrus	285	262	274	209	46.9	1030	98.24% with *C. mystusi*
C. aori	246	233	253	196	48.4	928	97.14% with *C. mystusi*
C. tripathii	128	124	122	109	47.8	483	98.51% with *C. geminus*
C. speratai	197	217	211	164	47.5	789	94.86% with *C. susanai*
C. falcatum	252	246	230	215	47.2	943	78.62% with *C. susanai*

with each other and confirming the distinction from other Dactylogyrids. Thus, the tree topologies further confirm our preliminary results.

Conclusion

As in previous molecular studies conducted on monogenoidean parasites, results showed that the 18S gene is a better potential marker than the 28S gene (Blair and Barker, 1993; Cunningham *et al*., 1995; Zhu *et al*., 1998; Matejusová *et al*., 2001) but in the present study, we have found both the markers useful for the characterization of parasites. It is, therefore concluded that molecular markers strongly demonstrate the genetic delineation and confirms the validation of *C. heterotylus*, *C. mystusi*, *C. proximus*, *C. geminus*, *C. agarwali*, *C. tukarami*, *C. bleekerai*, *C. susanai*, *C. gussevi*, *C. sclerovaginalis*, *C. longicirrus*, *C. aori*, *C. tripathii* n. sp., *C. indicus* n. sp., *C. speratai* n. sp. and *C. falcatum* n. sp. Thus, molecular study substantiated the morphological identity of different *Cornudiscoides* species and proves them genetically distinct species.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Agrawal, N., Verma, J., Rajvanshi, S. & Asthana, A. (2020). Application of two interesting statistical tools, used in differentiation of closely related species of Cornudiscoides Kulkarni, 1969 (Platyhelminthes: Monogenoidea: Dactylogyridae). *Uttar Pradesh Journal of Zoology,* 41(10) ,143-156.
2. Agrawal, N., Rajvanshi, S. & Verma, J. (2016). Two new species of the genus Cornudiscoides Kulkarni, 1969 from naked catfish Sperata *aor* (Hamilton, 1822): Specialist or Generalist? *Pakistan Journal of Zoology*, 48 (6) , 1687-1693.
3. Agrawal, N. & Vishwakarma, P. (1996). Six new species and re-description of two known species of the genus *Cornudiscoides* Kulkarni, 1969 (Monogenea) from Lucknow U.P. Indian Journal of Helmintology, 13 (1-2) , 10-31
4. Blair, D. & Barker, S.C. (1993). Affinities of the Gyliauchenidae: utility of 18S rRNA gene for phylogenetic inference in the Digenea (Platyhelminthes). *International Journal for Parasitology,* 23(4), 527–532. doi.org/10.1016/0020-7519(93)90042-W
5. Cunningham, C.O., Aliesky, H. & Collins, C.M. (2000). Sequence and secondary structure variation in the Gyrodactylus (Platyhelminth: Monogenea) ribosomal RNA gene array. *Journal of Parasitology,* 83 (3), 567–576.doi.org/10.1645/0022-3395(2000) 086[0567:SASSVI]2.0.CO;2
6. Cunningham, C.O., McGillivary, D.M. & Mackenze, K. (1995). Phylogenetic analysis of Gyrodactylus salaries Malmberg, 1957 based on the small subunit (18S) ribosomal RNA gene. *Molecular and Biochemical Parasitology,* 71(1) 139–142. doi: 10.1016/0166-6851(95)00043-z
7. Dayrat, B. (2005). Towards integrative taxonomy. *Biological Journal of Linnean Society*, 85(3) , 407–415. doi.org/10.1111/j.1095-8312.2005.00503.x
8. Devak, A. & Pandey, K.C. (2007). A new species of

Cornudiscoides Kulkarni, 1969 (Monogenoidea: Dactylogyridae) its locomotion, mode of attachment and distribution. *Indian Journal of Helminthology*, 25 (1-2), 41-58.

9. Dubey, A. Gupta, A.K. & Agrawal, S.M. (1992). Studies on monogenean parasites in freshwater fishes at Raipur IX. Two new species of the genus *Cornudiscoides* Kulkarni,1969 and a taxonomic discussion on species included in it. *Indian Journal of Helminthology*, 44, 109-115.
10. Ferguson, J. W. H. (2002). On the use of genetic divergence for identifying species. *Biological journal of the Linnean Society*, 75(4), 509-516.
11. Froese, R. and Pauly, D. (2014-2018) Fish base. World Wide Web electronic publication. Version 10/2015. Available at www.fishbase.org (accessed).
12. Gusev, A.V. (1976). Freshwater Indian Monogenoidea. Principles of systematics, analysis of the world faunas and their evolution. *Indian Journal of Helminthology*, 25 and 26, 1–241.
13. Hillis, DM., & Dixon, MT. (1991). Ribosomal DNA molecular evolution and phylogenetic inference. *Quarterly Review of Biology,* 66 (4), 411-453.doi: 10.1086/417338
14. Jayaram, K.C. (1955). Silurid fishes of India, Burma and Ceylon. XIV: Fishes of the genus Mystus Scopoli, 1777. *Record Indian Museum (Culcutta)*, 51, 527–558.
15. Kritsky, DC., Thatcher, VE. & Boeger, WA. (1986). Neotropical Monogenea 8. Reveision of *Urocleidoides* (Dactylogyridae, Ancyrocephalinae). *Proceding of Helminthological Society of Washington*, 53 (1) , 1-37.
16. Kulkarni, T. (1969). Studies on the monogenetic trematodes of fishes found in Hyderabad, Andhra Pradesh (India). Part I. *Riv di Parasitologica* 30 (2), 73–90.
17. Kumar, S., Stecher, G. & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology Evolution, 33(7) , 870-1874. doi:10.1093/molbev/msw054. doi: 10.1093/molbev/msw054.
18. Littlewood, D.T., Rohde, K. & Clough, K.A. (1998). The phylogenetic position of Udonella (Platyhelminth). *International Journal of Parasitology*, 28 (8), 1241-1250. doi: 10.1016/s0020-7519(98)00108-8.
19. Matejusová, I., Koubková, B., Amelio, S.D. & Cunniungham, C.O. (2001). Genetic characterization of six species of Diplozoon (Monogenea; Dioplozoiae*). Parasitology,* 123 (5), 465–474. doi: 10.1017/s0031182001008617.
20. Mollaret, I., Jamieson, B.G.M. & Justine, J.L. (2000). Phylogeny of Monopisthocotylea and Polyopisthocotylea (Platyhelminthes) inferred from 28S rDNA sequences. *International Journal of Parasitology*, 30 (2), 171–185. doi:10.1016/S0020-7519(99)00197-6.
21. Mollaret, I., Jamieson, B.G.M., Adlard, R.D., Hugall, A., Lecointre, G., Chombard, C. & Justine, J.L. (1997). Phylogenetic analysis of the Monogenea and their relationships with Digenea and Eucestoda inferred from 28S rDNA sequences. Molecular and Biochemical Parasitology, 90(2), 433–438. doi: 10.1016/S0166-6851(97)00176-X.
22. Nolan, M. & Cribb, T. (2005). The use and implication of ribosomal DNA sequencing for the discrimination of digenean species. *Advances in Parasitology,* 60,101-163. doi: 10.1016/S0065-308X(05)60002-4
23. Olson, P. & Littlewood D.T.G. (2002). Phylogeny of Monogenea – evidence from a medley of molecules. *International Journal for Parasitology*, 32(3), 233–244. doi.org/10.1016/S0020-7519 (01)00328-9
24. Olsen, G.J. & Woese, C.R. (1993). Ribosomal RNA: a key to phylogeny. *FASEB J* 7, 113-123. doi:10.1096/fasebj.7.1.8422957
25. Pandey, K.C. & Agrawal, N. (2008). An encyclopaedia of Indian Monogenoidea. New Delhi, Vitasta Publishing.
26. Plaisance, L., Littlewood, D.T.J., Olson, P.D. & Morand, S. (2005). Molecular phylogeny of gill monogeneans (Platyhelminthes, Monogenea, Dactylogyridae) and colonization of Indo-West Pacific butterflyfish hosts (Perciformes, Chaetodontidae). *Zoologica Scripta,* 34 (4), 425–436. doi.org/10.1111/j.1463-6409.2005.00191.x.
27. Rizvi, S.S.H. (1971). Monogenea of Pakistan fishes I. *Ancylodiscoides mystusi*, New species and *A. aori*, New species, from the gills of *Mystus aor* (Ham.). *Pakistan Journal of Zoology*, 3, (2)87-92.
28. Schlick-Steiner, B.C., Steiner, FM. Seifert, B. Stauffer, C. Christian, E. & Crozier, RH. (2010). Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology*, 55, 421–438. doi.org/10.1146/annurev-ento-112408-085432.
29. Šimková, A., Matejusová, I. & Cunniungham, C.O. (2006). A molecular phylogeny of the Dactylogyridae sensu Kristy and Boeger (1998) (Monogenea) based on the D1–D3 domain of larger subunit of rDNA. *Parasitology,* 133(1) , 43–53. doi: 10.1017/S0031182006009942.
30. Šimková, A., Plaisance, L., Matejusová, I., Morand, S. & Verneau, O. (2003). Phylogenetic relationships of the Dactylogyridae Bychowsky, 1933 (Monogenea: Dactylogyridea): the need for the systematic revision of the Ancyrocephalinae Bychowsky, 1937. *Systematic Parasitology*, 54(1), 1–11.doi.org/10.1023/A:1022133608662.
31. Shrivastava, R.R., Agrawal, N. & Upadhyay, M.K. (2013). Molecular analysis based on 28S rDNA of Dactylogyroides species parasitizing *Puntius* species. *Bioengineering and Biosciences*, 1(3), 25–30. doi: 10.13189/bb.2013.010301
32. Van Steenberge, M., Pariselle, A., Huyse, T., Volckaert, F. A., Snoeks, J., & Vanhove, M. P. (2015). Morphology, molecules, and monogenean parasites: an example of an integrative approach to cichlid biodiversity. *PloS one*, 10, e0124474. doi.org/10.1371/journal.pone.0124474
33. Verma, J., Agrawal, N. & Verma, A.K. (2016). The use of large and small subunits of ribosomal DNA in evaluating phylogenetic relationships between species of *Cornudiscoides* Kulkarni, 1969 (Monogenoidea: Dactylogyridae) from India*. Journal of Helminthology,* 91(2) , 206-214. doi: 10.1017/S0022149X16000134
34. Verma J., Rajvanshi S. & Agrawal, N. (2018). Genetic characterization of three species of the genus*Cornudiscoides* Kulkarni, 1969 (Monogenoidea: Dactylogyridae), Parasitizing long whiskered cat fish *Sperata aor* (Ham) using ribosomal and mitochondrial DNA. *Journal of Zoological Sciences*, 6, 31-37.
35. Zhu, X., Gasser, R.B. & Chilton, N.B. (1998). Differences in the 5.8S rDNA sequences among ascarid nematodes. *International Journal for Parasitology,* 28(4), 617–622. doi.org/10.1016/S0020-7519(97)00214-2.