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Chelatrematidae n. fam., a new family of digenetic trematodes from the South Western Ghats, India, erected on the basis of morphological and molecular studies

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Abstract

On the basis of the morphological characterization of *Chelatrema neilgherriensis* Manjula & Janardanan, 2006 recovered from the freshwater fish *Barilius gatensis* (Valenciennes, 1844) in the Wayanad region of the Western Ghats, the diagnostic features of the genus *Chelatrema* Gupta & Kumari, 1973 have been modified. Based on the phylogenetic analysis of *C. neilgherriensis* and comparative morphology studies relative to members of other families of Gorgoderoidea Looss, 1901, this genus is placed in a new family Chelatrematidae n. fam. The studies revealed the molecular and morphological closeness of *Chelatrema* with *Paracreptatrematina limi* Amin & Myer, 1982, and the latter is transferred to this new family. Hence the new family Chelatrematidae n. fam. comprises the genera *Chelatrema* and *Paracreptatrematina*.

Introduction

The composition of the superfamily Gorgoderoidea Looss, 1899 is always contentious. According to Bray & Blair (2008) there are no outstanding morphological autapomorphies for this superfamily. The hosts and sites of infection vary greatly within this group and it can be found virtually in all vertebrate groups from elasmobranchs to mammals.

According to Odening (1974) this superfamily contained only a single family, Gorgoderidae Looss, 1899. Later the molecular phylogenetic analyses of Trematoda by Olson *et al.* (2003) added seven more families to the superfamily Gorgoderoidea: Callodistomidae Odhner, 1910; Dicrocoeliidae Looss, 1899; Encyclometridae Mehra, 1931; Haploporidae Nicoll, 1914 (with Atractotrematidae, Yamaguti, 1931 nested within it); Orchipedidae Skrjabin, 1913; Paragonimidae Dollfus, 1939; and Troglotrematidae Odhner, 1914. Curran *et al.* (2006) opined that the superfamily Allocreadioidea Looss, 1902 should be treated as a junior synonym of Gorgoderoidea and Allocreadiidae Looss, 1902 should, therefore, come under Gorgoderoidea. At the same time the families Haploporidae and Atractotrematidae which were included earlier (Olson *et al.*, 2003) under Gorgoderoidea should be recognized in a separate superfamily Haploporoidea. Choudhury *et al.* (2007) pointed out that Allocreadiidae is closely associated with Callodistomidae and Gorgoderidae. Bray & Blair (2008) followed the classification proposed by Olson *et al.* (2003) but for 'convenience of identification' several other families, Anchitrematidae Mehra, 1935, Brachycoeliidae Looss, 1899, Braunotrematidae Yamaguti, 1958, Collyriclidae Ward, 1917, Cortrematidae Yamaguti, 1958, Mesocoeliidae Dollfus, 1929 and Prouterinidae Foreyt, Schell & Beyer, 1996, were added to Gorgoderoidea on the basis of morphological similarities to the families recognized through molecular studies. Later, Heneberg & Literák (2013) transferred the family Collyriclidae to the superfamily Microphalloidea Ward, 1901 based on phylogenetic analyses of 18S and 28S rDNA sequences. According to Kanarek *et al.* (2014) the family Cortrematidae should be considered among the synonyms of Pleurogenidae Looss, 1899. Tkach *et al.* (2018) synonymized the family Anenterotrematidae Yamaguti, 1958 with Dicrocoeliidae. More molecular studies are required to understand and establish the accurate taxonomic positions of these parasite groups.

The genus *Chelatrema* Gupta & Kumari, 1973 was erected for *Chelatrema smythi* Gupta & Kumari, 1973 from the freshwater fish, *Chela bacala* (Hamilton, 1822) of Ropar, India. Gupta & Kumari (1973) described this genus as a member of the family Hemiuridae Looss, 1899, as it possesses a number of characters relevant to the family. Later, Manjula & Janardanan (2006) described a new species, *Chelatrema neilgherriensis* Manjula & Janardanan, 2006, from freshwater fishes of Noolpuzha river, Kerala, India. Campbell (2008) stated that *Chelatrema* appears to be a member of the family Gorgoderidae Looss, 1899, but should be considered as genus *Inquirendum*, of Gorgoderidae, pending further study. In the present study we provide the first molecular data for a member of *Chelatrema* – *C. neilgherriensis* from *Barilius gatensis* (Valenciennes, 1844) in the Wayanad region of the Western Ghats, India and the results of the studies on phylogenetic relationships of this genus with other members of the superfamily Gorgoderoidea Looss, 1901. On this basis, a new family, Chelatrematidae n. fam., is proposed to accommodate the genus *Chelatrema*.

Material and methods

Isolation and study of parasite

Live specimens of *B. gatensis* were collected from water bodies of Wayanad (between North 11′27′ and 15′58′ and East 75′47′ and 70′27′), Kerala, India. The specimens were dissected in physiological saline (0.75% sodium chloride solution) under a Labomed (Luxeo 4Z) stereozoom microscope. Parasites were transferred to a Petri dish containing saline, and live parasites (both unstained and neutral red-stained) were observed under a Nikon ECLIPSE Ni–U phase contrast Research Microscope (Japan) to study the morphological characteristics. Permanent whole mounts were prepared by fixing them in 5% formalin under slight cover glass pressure and staining with acetocarmine following Cantwell (1981). The infection parameters such as prevalence and mean intensity were calculated following Bush *et al.* (1997). Photographs were taken with Nikon Y-TV55 camera and Nikon NIS Elements imaging software attached to the microscope. Figures were drawn with Nikon Y-IDT drawing tube and measurements (in μm) with mean in parentheses were taken with Nikon NIS Elements imaging software.

DNA extraction, amplification and sequencing

A single adult specimen preserved in 96% ethanol was used for molecular analysis (table 1). Total DNA was extracted from the individual fluke using a 'hot shot' technique (Truett, 2006). Ribosomal 28S rRNA gene fragment was amplified with the primers 28S_A (5′-TCG ATT CGA GCG TGA WTA CCC GC-3′) and 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) (Tkach *et al.*, 2003; Matejusova & Cunningham, 2004). Initial polymerase chain reaction (PCR) was performed in a total volume of 25 μl containing 0.25 mM of each primer pair, 25 mg of total DNA in water, 12.5 μl GoTaq Green Master mix (Promega, Madison, Wisconsin, USA). Amplification of a 1200-base pairs (bp) fragment of 28S rRNA gene was performed in a GeneAmp 9700, Applied Biosystems (Waltham, Massachusetts, USA), with a 5-min denaturation at 96 °C, 35 cycles of 1 min at 96 °C, 20 s at 55 °C and 2 min 30 s at 72 °C, and a 7-min extension at 72 °C. Negative and positive controls using both primers were used. PCR products were directly sequenced using an ABI Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, Massachusetts, USA), as recommended by the manufacturer, with the internal sequencing primers described by Tkach *et al.* (2003). PCR product sequences were analysed using a GA3500 (Applied Biosystems, Waltham, Massachusetts, USA) genetic analyser at the Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS. The sequence was submitted to the GenBank database (ON493538).

Alignments and phylogenetic analysis

Ribosomal DNA sequences were assembled with SeqScape v.2.6 software, provided by Applied Biosystems (Waltham, Massachusetts, USA). Alignments and estimations of the number of variable sites and sequence differences were performed using the MEGA 7.1 software (Kumar *et al.*, 2016). Phylogenetic analysis was performed using the Bayesian algorithm with the MrBayes v. 3.1.2 software (Huelsenbeck *et al.*, 2001). The best nucleotide substitution model, TVM + I+G (Posada, 2003) was estimated with jModeltest v. 2.1.5 software (Darriba *et al.*, 2012). Bayesian analysis was performed using 10,000,000 generations with two independent runs. Summary parameters and the phylogenetic tree were calculated with a burn-in of 25% of generations. The significance of the phylogenetic relationships was estimated using posterior probabilities (Huelsenbeck *et al.*, 2001). GenBank sequence data for representatives of Gorgoderoidea Looss, 1901 and out group taxa used in molecular analysis, including references and accession numbers are given in table 1.

Results

Chelatrema neilgherriensis Manjula & Janardanan, 2006 (fig. 1)

Type host: *Devario neilgherriensis* (Day, 1867), Cyprinidae Ranifesque, 1815.

Other hosts: Labeo rohita (Hamilton, 1822), *B. gatensis*, Cyprinidae.

Site of infection: intestine.

Locality: Niravilpuzha, Varadimoola, Valavayal, Thirunelli, Periya and Makkimala of Wayanad region.

Period of collection: March – April 2019, July 2019 and November 2019.

Prevalence of infection: 12 of 48 (25.00%) *B. gatensis* examined.

Mean intensity of infection: 1.66 (20 parasites from 12 infected fishes)

Adult worm (based on 18 specimens): elongate body, aspinose, slightly pink, 1375–4976 × 583–2140 (2792 × 1074). Diffused eye spot pigments present in immature specimens, lateral to oesophagus. Oral sucker subterminal, round 234–527 × 217–504 (364 × 359). Ventral sucker round, larger than oral sucker, 228–897 × 237–868 (502 × 489), 354–1289 (797) from oral sucker. Pharynx muscular, 65–153 × 65–153 (102 × 98). Oesophagus 74–322 × 15–134 (164 × 74). Intestinal bifurcation anterior to ventral sucker; caeca terminate near posterior extremity, 1055–3709 × 50–254 (1896 × 109). Excretory bladder I-shaped, extends to level of testes. Two testes: left testis 49–276 × 33–262 (150 × 117) and right testis 45–261 × 31–294 (134 × 113). Cirrus-sac anterior to ventral sucker, post-bifurcal, medially placed; containing bipartite seminal vesicle and ejaculatory duct; 70–191 × 37–107 (144 × 80). Genital pore lateral. Ovary posterior to ventral sucker 66–218 × 61–235 (120 × 107). Uterine seminal receptacle round, lateral to ovary. Vitellerium single compact mass. Uterus fills testicular region, extends extra-caecal up to level of intestinal bifurcation. Metraterm opens at genital pore. Eggs numerous, round to oval, embryonated, 11–63 × 6–44 (34 × 22).

Remarks: *Chelatrema neilgherriensis* was first described by Manjula & Janardanan (2006) from freshwater fishes, *Danio neilgherriensis* (Day, 1867) and *L. rohita* (Hamilton, 1822), of Wayanad as the second species of the previously monotypic *Chelatrema*. In the present study, adult worms were collected from a new host, *B. gatensis*, from the same region. The specimens

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Table 1. List of taxa, incorporated into 28S rDNA based molecular analysis (*n* – number of sequences).

Species	*n*	Host species	Locality	Reference	Accession number
Allocreadiidae					
Acrolichanus auriculatus	1	*Acipenser schrenckii*	Russia, Irtysh River	Atopkin *et al.*, 2020	MN524581
Allocreadium apokryfi	1	*Labeobarbus aeneus*	South Africa: Vaal River	Dos Santos *et al.* (2021)	MW907594
Allocreadium hemibarbi	1	*Hemibarbus labeo*	Russia: Primorsky territory	Vainutis (2020)	MK211220
Allocreadium isoporum	1	*Barbatula barbatula*	Russia, IL'd River, upper Volga River basin	Petkevičiūtė *et al.* (2018)	MH143102
Allocreadium khaikanensis	1	*Rhynchocypris oxycephalus*	Russia, Poperechny spring (Komissarovka River basin)	Vainutis (2020)	MK211211
Allocreadium lobatum	1	*Semotilus corporalis*	United States, Maine, Moosehead L.	Curran *et al.* (2006)	EF032693
Allocreadium neotenicum	1	*Pisidium* sp.	Norway, Lake Nordersjoen	Petkevičiūtė *et al.* (2018)	MH143105
Allocreadium gotoi	1	*Misgurnus anguillicaudatus*	Japan, Nagano, Iiyama, Midori R.	Shimazu (2017)	LC215274
Allocreadium sp. YLF1	1	*Schizothorax parvus*	China		MN969626
Allocreadium sp. YLF1	1	*Schizothorax yunnanensis*	China		MN969627
Allocreadium sp.	1	*Sphaerium corneum*	Ukraine, River Belka (Dnieper River basin)	Petkevičiūtė *et al.* (2010)	GU462121
Allocreadium sp. 1	1	*Phoxinus phoxinus*	Russia, Razdolnaya River	Vainutis (2020)	MK211209
Allocreadium sp.	1	*Carassius carassius*	Russia, Arsenyavka River		MK258685
Auriculostoma astyanace	1	*Astyanax fasciatus*	Costa Rica, Guanacaste, Rio Sapoa	Razo-Mendivil *et al.* (2014)	KF631422
Auriculostoma lobata	1	*Brycon guatemalensis*	Mexico, Tenosique, El Managal Lagoon	Hernández-Mena *et al.* (2016)	KX954172
Auriculostoma tica	1	*Gymnotus maculosus*	Costa Rica: Creek at Pitaya, Guanacaste	Hernández-Mena *et al.* (2019)	MH997001
Auriculostoma totonacapanensis	1	*Astyanax aeneus*	Mexico: Metzabok, Chiapas	Pérez-Ponce de León & Hernández-Mena (2019)	MK648262
Auriculostoma sp.	1	*Astyanax mexicanus*	Mexico: Filipinas, Veracruz	Razo-Mendivil *et al.* (2014)	KF631417
Bunodera luciopercae	1	*Perca fluviatilis*	Russia, River Tvertsa, upper Volga River basin	Petkevičiūtė *et al.* (2010)	GU462124
Bunodera vytautasi	1	*Pungitius pungitius*	Russia, Okhotiya, Chyornoe L.	Atopkin *et al.* (2018)	MG262545
Crepidostomum farionis	1	*Salvelinus alpinus*	Iceland, Lake Hafravatn	Faltýnkova *et al.* (2020)	MT080780
Crepidostomum metoecus	1	*P. casertanum*	Norway, County Troms, Takvatn L.	Soldánová *et al.* (2017)	KY513148
Crepidostomum oshmarini	1	*Cottus gobio*	Russia: River IL'd, upper Volga River basin	Petkevičiūtė *et al.* (2018)	MH159989
Crepidostomum sp. 1	1	*Sphaerium* sp.	Norway, County Troms, Takvatn Lake	Soldánová *et al.*, 2017	KY513149
Crepidostomum sp. 2	1	*Siphlonurus lacustris*	Norway, County Troms, Takvatn Lake	Soldánová *et al.* (2017)	KY513151
Crepidostomum sp. 2	3	*P. casertanum*	Russia, Crimea, River Burulcha	Petkevičiūtė *et al.* (2018)	MH143117-119
Crepidostomum affine	1	*Aplodinotus grunniens*	United States, Mississippi, Pearl River	Tkach *et al.* (2013)	KF356363
Crepidostomum illinoiense	1	*Hiodon alosoides*	United States, Red Lake River	Curran *et al.* (2011)	HQ833705
C. illinoiense	1	*H. alosoides*	United States: Red Lake River, Minnesota	Tkach *et al.* (2013)	KF356372

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Table 1. (*Continued.*)

Species	*n*	Host species	Locality	Reference	Accession number
Crepidostomum cornutum	1	*Lepomis gulosus*	United States, Mississippi, Pascagoula River	Curran *et al.* (2006)	EF032695
Crepidostomum auritum	1	*Aplodinotus grunniens*	United States, Mississippi, Pearl River	Tkach *et al.* (2013)	KF356373
Creptotrema funduli	1	*Fundulus notatus*	United States, Mississippi, Harrison County, Biloxi River	Curran *et al.* (2012)	JQ425256
Creptotrematina aquirrepequenoi	3	*Astyanax aeneus*	Costa Rica, Rio Tempisquito	Curran *et al.* (2011)	HQ833708–HQ833710
Margotrema bravoae	1	*Characodon audax*	Mexico	Pérez-Ponce de León *et al.* (2016)	KT833275
Paracreptotrema blancoi	1	*Priapichthys annectens*	Costa Rica, Río Orosí	Pérez-Ponce de León *et al.* (2016)	KT833285
Pseudoparacreptotrema profundulusi	1	*Profundulus* sp.	Mexico, San Juan del Río, Oax, Río Templo	Pérez-Ponce de León *et al.* (2016)	KT833291
Pseudoparacreptotrema macroacetabulata	1	*Profundulus punctatus*	Guatemala, Río Primavera	Pérez-Ponce de León *et al.* (2016)	KT833316
Wallinia anindoi	1	*Astyanax aeneus*	Guatemala: Rio las Cabezas at Sanarate, El Progreso	Hernández-Mena *et al.* (2019)	MH997015
Wallinia caririensis	1	*Astyanax bimaculatus*	Brazil, Batateiras River, Ceará state	Da Silva *et al.* (2021)	MW024899
Wallinia chavarriae	1	*Astyanax aeneus*	Costa Rica, Guanacaste, Rio Animas	Curran *et al.* (2011)	HQ833703
Callodistomidae					
Prosthenhystera caballeroi	1	*Astyanax aeneus*	Nicaragua: Rio Quezalguaque near Telica	Tkach & Curran (2015)	KM871186
Prosthenhystera gattii	1	*Bryconamericus ikaa*	Argentina	Montes *et al.* (2020)	MF664223
Prosthenhystera obesa	1	*Ictalurus punctatus*	United States	Curran *et al.* (2006)	EF032690
Prosthenhystera oonastica	1	*I. furcatus*	United States: Pearl River, Mississippi	Tkach & Curran (2015)	KM871180
Chelatrematidae n. fam.					
Chelatrema neilgherriensis	1	*Barilius gatensis*	India	present study	not applicable (n/a)
Paracreptotrematina limi	1	*Umbra limi*	United States: Cabela's store in East Grand Forks, Minnesota	Curran *et al.* (2011)	HQ833706
Dicrocoeliidae					
Brachydistomum olssoni	1	*Apus apus*	Czech Republic: Prague		KU563712
Brachydistomum sp.	1	*Prunella rubeculoides*	Pakistan	Suleman *et al.* (2020)	MK685272
Brachylecithum capilliformis	1	*Locustella fluviatilis*	Czech Republic: Central Moravia	Hildebrand *et al.* (2016)	KU212184
Brachylecithum glareoli	1	*Myodes glareolus*	Brazil	Hildebrand *et al.* (2016)	KU212201
Brachylecithum grummti	1	*Attila cinnamomeus*	Brazil: Para State, Gurupi Nature Reserve	Hildebrand *et al.* (2015)	KP765768
Brachylecithum kakea	1	*Acrocephalus arundinaceus*	Czech Republic: Central Moravia	Hildebrand *et al.* (2016)	KU212178
Brachylecithum laniicola	1	*Lanius collurio*	Czech Republic: Central Moravia	Hildebrand *et al.* (2016)	KU212183
Brachylecithum strigis	1	*Otus scops*	Czech Republic: Central Moravia	Hildebrand *et al.* (2016)	KU212195
Dicrocoelium dendriticum	1	*Marmota bobak*	Ukraine, Kharkiv Region	Tkach *et al.* (2000)	AF151939
Dicrocoelium hospes	1	n/a	n/a		AY251233

Dicrocoeliidae sp. 2	1	*Bradybaena similaris*	Japan	Waki *et al.* (2018)	MG822659
Dicrocoeliidae sp. 3	1	*Phaedusa gouldi*	Japan	Waki *et al.* (2018)	MG822662
Dicrocoeliidae sp. 4	1	*Megalophaedusa decussata*	Japan	Waki *et al.* (2018)	MG845907
Dicrocoeliidae sp. 5	1	*Discus pauper*	Japan	Waki *et al.* (2018)	MG845913
Eurytrema pancreaticum	1	'sheep'	China, Qiqihaer, Heilongjiang Province	Su *et al.* (2018)	KY490004
Lutztrema attenuatum	1	*Turdus philomelos*	Czech Republic: Zahlinice, Hulin, district, Kromeriz		KU563714
Lutztrema microacetabulare	1	*Hipposideros armiger*	Vietnam	Tkach *et al.* (2018)	MH158562
Lutztrema microstomum	1	*Cyanocitta cristata*	United States: near Grand Forks, Grand Forks County, North Dakota	Hildebrand *et al.* (2015)	KP765765
Lutztrema monenteron	1	*Turdus migratorius*	United States: near Grand Forks, Grand Forks County, North Dakota	Hildebrand *et al.* (2015)	KP765766
Lutztrema sp.	1	*Sylvia atricapilla*	Czech Republic	Aldhoun *et al.* (2018)	MG560859
Lutztrema sp.	1	*Acrocephalus arundinaceus*	Czech Republic	Hildebrand *et al.* (2015), unpublished	KT387689
Lyperosomum clathratum	1	*Apus apus*	Czech Republic	Hildebrand *et al.* (2019)	MK478493
Lyperosomum cuauhxinqui	1	*Melanerpes aurifrons*	Mexico: Rio Purificacion, Tamaulipas	González-García *et al.* (2020)	MT340826
Lyperosomum intermedium	1	*Oryzomys palustris*	United States: Florida	Tkach *et al.* (2018)	MH158563
Lyperosomum longicauda	1	*Dendrocitta vagabunda*	Pakistan	Suleman *et al.* (2020)	MK685270
Lyperosomum petiolatum	1	*Ligia* sp.	Poland	Hildebrand *et al.* (2019)	MK618580
Lyperosomum sarothrurae	1	*Sarothrura pulchra*	Uganda: Kibale National Park	Hildebrand *et al.* (2015)	KP765767
Lyperosomum sp.	2	*Turdus merula*	Czech Republic	Aldhoun *et al.* (2018)	MG560864
Lyperosomum sp.		*Turdus philomelos*	Czech Republic	Aldhoun *et al.* (2018)	MG560865
Lyperosomum sp. 1	1	*Acrocephalus arundinaceus*	Czech Republic	Hildebrand *et al.* (2019)	MK496656
Lyperosomum sp. 2	1	*Delichon urbicum*	Czech Republic	Hildebrand *et al.* (2019)	MK496657
Lyperosomum sp. 2	1	*Delichon urbicum*	Czech Republic	Hildebrand *et al.* (2019)	MK626682
Lyperosomum transcarpathicus	1	n/a	n/a	Tkach *et al.* (2001)	AF151943
Lyperosomum cf. *turdia*	1	*Turdus merula*	Poland	Hildebrand *et al.* (2019)	MK478486
Metadelphis cesartapiai	1	*Anoura peruana*	Ecuador: Reserva Integral de Bosque Otonga	Achatz *et al.* (2020)	MT227171
Metadelphis sp.	1	*Lonchophylla robusta*	Panama	Tkach *et al.* (2018)	MH158567
Parametadelphis compactus	1	*Lonchophylla handleyi*	Peru	Tkach *et al.* (2018)	MH158569
Parametadelphis sp.	1	*Pteronotus parnellii*	Mexico: Isla Don Panchito, Jalisco	Pérez-Ponce de León & Hernández-Mena (2019)	MK648279
Platynosomum illiciens	1	*Hemidactylus mabouia*	Brazil: Fortaleza, Ceará	Pinto *et al.* (2018)	MH156569
Pojmanskatrema balcanica	1	*Neomys fodiens*	Bulgaria	Hildebrand & Tkach (2019)	MK426285

(*Continued*)

Table 1. (*Continued.*)

Species	*n*	Host species	Locality	Reference	Accession number
Skrjabinus kalmikensis	1	*Delichon urbicum*	Czech Republic	Hildebrand *et al.* (2019)	MK478495
Stromitrema koshewnikowi	1	*Hirundo rustica*	Czech Republic	Hildebrand *et al.* (2019)	MK474483
Zonorchis alveyi	1	*Zonotrichia albicollis*	United States	Hildebrand *et al.* (2019)	MK480327
Zonorchis delectans	1	*Caryothraustes poliogaster*	Costa Rica	Hildebrand *et al.* (2019)	MK480329
Zonorchis sp.	1	*Phaenostictus mcleannani*	Costa Rica	Hildebrand *et al.* (2019)	MK480328
Encyclometridae					
Encyclometra colubrimurorum	1	n/a	n/a	Tkach *et al.* (2001)	AF184254
Otongatrema caenolestesi	1	*Caenolestes fuliginosus*	Ecuador: Reserva Integral de Bosque Otonga, Cotopaxi Province	Achatz *et al.* (2020)	MT227169
Polylekithum catahoulensis	1	*Ictalurus furcatus*	United States	Curran *et al.* (2006)	EF032698
Polylekithum ictaluri	1	*I. furcatus*	United States	Curran *et al.* (2006)	EF032697
Gordoderidae					
Anaporrhutum sp.	1	*Chiloscyllium punctatum*	Australia	Cutmore *et al.* (2013)	KF013184
Degeneria halosauri	1	*Halosauropsis macrochir*	North-eastern Atlantic Ocean	Olson *et al.* (2003)	AY222257
Gorgodera cygnoides	1	*Pelophylax ridibundus*	Bulgaria	Olson *et al.* (2003)	AY222264
Gorgoderina lufengensis	1	*Nanorana yunnanensis*	China		MH277507
Nagmia floridensis	1	*Rhinoptera bonasus*	United States	Olson *et al.* (2003)	AY222262
N. floridensis	1	*Dasyatis sabina*	United States	Curran *et al.* (2006)	EF032691
Nagmia sp.	1	*Stegostoma fasciatum*	Australia	Cutmore *et al.* (2013)	KF013192
Nagmia sp.	1	*Stegostoma fasciatum*	Australia	Cutmore *et al.* (2013)	KF013194
Phyllodistomum angulatum	1	*Sander lucioperca*	Russia, Rybinsk Water Reservoir	Stunžėnas *et al.* (2017)	KX957735
P. angulatum	1	*Lota lota*	Russia, Rybinsk Water Reservoir	Stunžėnas *et al.* (2017)	KX957733
Phyllodistomum brevicecum	2	*U. limi*	Canada	Razo-Mendivil *et al.* (2013)	KC760206-07
Phyllodistomum centropomi	1	*Centropomus parallelus*	Mexico, Tlacotalpan, Veracruz	Pérez-Ponce de León *et al.* (2015)	KM659384
Phyllodistomum folium	1	*G. cernua*	Lithuania, Curonian Lagoon	Stunžėnas *et al.* (2017)	KX957729
P. folium	1	*Rutilus rutilus*	Russia, Sunoga River	Petkevičiūtė *et al.* (2020)	MT872645
P. folium	1	*Scardinius erythrophthalmus*	Russia, Rybinsk Water Reservoir	Petkevičiūtė *et al.* (2020)	MT872646
Phyllodistomum hoggettae	1	*Plectropomus leopardus*	Australia	Cutmore *et al.* (2013)	KF013191
P. hoggettae	1	n/a	n/a		MG722710
Phyllodistomum hyporhamphi	1	*Hyporhamphus australis*	Australia	Cutmore *et al.* (2013)	KF013190
Phyllodistomum inecoli	1	*Profundulus punctatus*	Mexico, Tlacotalpan, Veracruz	Pérez-Ponce de León *et al.* (2015)	KM659387

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P. inecoli	1	*Profundulus* sp.	Mexico, Tlacotalpan, Veracruz	Pérez-Ponce de León *et al.* (2015)	KM659389
Phyllodistomum kanae	1	*Hynobius retardatus*	Japan: Hokkaido, Pippu	Nakao (2015)	AB979868
Phyllodistomum kupermani	2	*Perca fluviatilis*	Russia, Rybinsk Water Reservoir	Petkevičiūtė *et al.* (2020)	MT875008-09
Phyllodistomum lacustri	1	*Ameiurus melas*	United States	Curran *et al.* (2006)	EF032692
Phyllodistomum macrocotyle	1	*Dreissena polymorpha*	Belarus, Lake Lepelskoe	Stunžėnas *et al.* (2004)	AY288828
P. macrocotyle	1	*Leuciscus idus*	Russia, Rybinsk Water Reservoir	Petkevičiūtė *et al.* (2020)	MT872663
P. macrocotyle	1	*Scardinius erythrophthalmus*	Russia, Rybinsk Water Reservoir	Petkevičiūtė *et al.* (2020)	MT872664
Phyllodistomum magnificum	1	*Tandanus tandanus*	Australia	Cutmore *et al.* (2013)	KF013189
Phyllodistomum pacificum	1	*Pantolabus radiatus*	Australia: Moreton Bay, Queensland	Cutmore & Cribb (2018)	MG845599
Phyllodistomum pseudofolium	1	*Pisidium amnicum*	Russia: a pond near Rybinsk water reservoir on the Volga River	Stunžėnas *et al.* (2017)	KX957730
Phyllodistomum spinopapillatum	1	*Profundulus balsanus*	Mexico, Tlacotalpan, Veracruz	Pérez-Ponce de León *et al.* (2015)	KM659381
Phyllodistomum cf. *symmetrorchis*	1	*Clarias gariepinus*	Kenya	Cutmore *et al.* (2013)	KF013174
Phyllodistomum umblae	1	*Salvelinus alpinus*	Iceland, Lake Hafravatn	Faltýnkova *et al.* (2020)	MT076069
Phyllodistomum vaili	1	*Mulloidichthys vanicolensis*	Australia	Cutmore *et al.* (2013)	KF013187
Pseudophyllodistomum anguilae	1	n/a	China		MG976846
Pseudophyllodistomum johnstoni	1	*Macrobrachium australiense*	Australia	Cutmore *et al.* (2013)	KF013182
Staphylorchis cymatodes	1	*Sphyrna lewini*	Australia	Cutmore *et al.* (2010)	HM486319
Xystretrum solidum	1	*Balistes vetula*	Mexico: Puerto Morelos, Quintana Roo	Pérez-Ponce de León & Hernández-Mena (2019)	MK648284
Monorchidae					
Cableia pudica	1	*Cantherhines pardalis*	Australia	Olson *et al.* (2003)	AY222251
Orchipedidae					
Orchipedum tracheicola	1	*Cygnus olor*	United Kingdom: Scotland	Olson *et al.* (2003)	AY222258
			OUTGROUP		
Skrjabinopsolus nudidorsalis	1	*Acipenser ruthenus*	Russia, Oka River	Sokolov *et al.* (2020)	MN700998

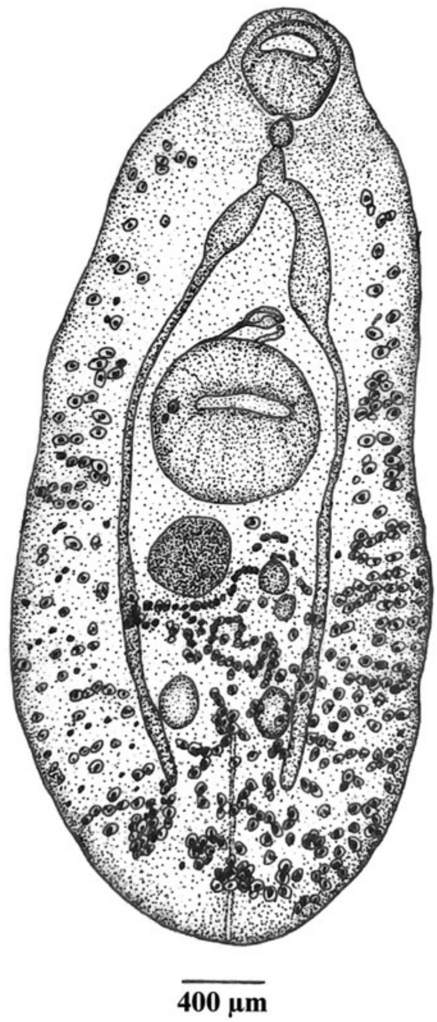

Fig. 1. *Chelatrema neilgherriensis* Manjula & Janardanan, 2006.

agree well with *C. neilgherriensis* in the presence of uterine coils up to the level of caecal bifurcation, presence of diffused eye spot pigments, sucker length ratio and shape of eggs. At the same time, there are discrepancies in morphometric parameters of our worms and *C. neilgherriensis* from the first description (table 2). Nevertheless, based on location and high morphological similarities we considered the worms from our study to be *C. neilgherriensis*.

Genus *Chelatrema* Gupta & Kumari, 1973

Diagnosis: elongate body, medium to large size; tegument thick and smooth; diffused eye spot pigments present or absent. Oral sucker subterminal. Ventral sucker larger than oral sucker, pre-equatorial. Pharynx well developed. Oesophagus short. Caeca terminating near posterior extremity. Two testes, diagonal to symmetrical, in hindbody, separated by uterus. Cirrus-sac short, median, postbifurcal, anterior to ventral sucker, enclosing seminal vesicle and cirrus. Genital pore postbifurcal, submedian, slightly anterior to ventral sucker. Ovary pre-testicular, submedian. Uterine seminal receptacle present. Uterus strongly convoluted in intercaecal and extracaecal areas and extends to posterior extremity; sometimes extracaecal up to caecal bifurcation. Vitellarium single, compact, slightly lobed mass, submedian, lateral to ovary. Eggs small, round to oval, numerous, embryonated. Excretory vesicle I-shaped; pore terminal. Intestinal parasites of freshwater fishes, India. Type-species *Chelatrema smythi* Gupta & Kumari, 1973.

Remarks: the genus *Chelatrema* was proposed in the family Hemiuridae Looss, 1899. Campbell (2008) stated that it should be considered as a gorgoderid genus *inquirendum* until it is re-examined. The study of Manjula & Janardanan (2006) pointed out similarities and differences of *C. neilgherriensis* with *C. smythi*. The notable morphological differences are the presence of diffuse eye spots and extent of uterine coils (which extend extracaecally up to the level of caecal bifurcation) in *C. neilgherriensis*. Therefore, the diagnosis of *Chelatrema* was corrected and presented here.

Phylogenetic analysis

A 1261 bp fragment of the 28S rRNA gene was successfully generated for a single specimen of *C. neilgherriensis*. Alignment of all available 28S rDNA sequences of Gorgoderoidea allows a 933 bp fragment for phylogenetic analysis. Results of Bayesian phylogenetic analysis showed clustering of trematodes according to familial membership within a monophyletic Gorgoderoidea (fig. 2). *Chelatrema neilgherriensis* was closely related to *Paracreptatrematina limi* Amin & Myer, 1982; these two species formed a clade, sister to Dicrocoeliidae and Encyclometridae. The genetic *P*-distance value between *C. neilgherriensis* and *P. limi* was 8.67 ± 0.84% which corresponds to internal mean values for most gorgoderoid families, calculated on the basis of the available data set: 6.73 ± 0.44% for Allocreadiidae; 7.52 ± 0.47% for Dicrocoeliidae; and 8.75 ± 0.7% for Encyclometridae Mehra, 1931. An extreme minimum mean value was observed for Callodistomidae Odhner, 1910 (2.64 ± 0.41%); we omit these data because of lack of representative molecular data for this family. Genetic *P*-distances between different families within Gorgoderoidea from our study ranged from 12.15 ± 0.88% (Allocreadiidae/Callodistomidae) to 20.45 ± 1.05% (Gorgoderidae/Callodistomidae), considerably higher than the *P*-distance value between *C. neilgherriensis* and *P. limi*. Within Gorgoderidae, mean *P*-distance value by means of 28S rDNA sequence data was 13.52 ± 0.6%, corresponding to interfamilial divergence level. Accepting that the Gorgoderidae clade showed internal differentiation into four highly supported and divergent subclades, this family represents a gorgoderoid group requiring comprehensive studies taxonomically and phylogenetically. On the basis of our molecular results, we propose that *C. neilgherriensis* and *P. limi* represent members of a new family of Gorgoderoidea.

Family Chelatrematidae n. fam.

Elongate body, fusiform, medium to large; tegument thick, smooth; eye spot pigments present or absent. Suckers well developed. Oral sucker subterminal, with or without lobes. Ventral sucker equal or larger than oral sucker, pre-equatorial. Pharynx well developed. Oesophagus short. Caeca extend to near posterior extremity. Testes diagonal, symmetrical, in hindbody, separated by uterus. Cirrus-sac small, postbifurcal, median or lateral to ventral sucker, enclosing seminal vesicle and cirrus. Genital pore median or lateral, slightly anterior to ventral sucker. Ovary submedian, pre-testicular, posterior to ventral sucker. Seminal receptacle present. Uterus extends to posterior extremity, intercaecal or extracaecal; sometimes extracaecal up to caecal bifurcation. Vitellarium variable, single compact mass or small follicles in

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Table 2. Morphological and morphometric comparison of original description of *Chelatrema neilgherriensis* Manjula & Janardanan, 2006 with worms obtained in the present study.

Characters	*C. neilgherriensis* (original description)	*C. neilgherriensis* (present study)
body	large, red, elongate, ovoid, 2714–6500 × 1011–2602 (4413 × 1747)	elongate, aspinose, slight pink, large, eyespot pigment in fore-body, 1375–4976 × 583–2140 (2792 × 1074)
oral sucker	round, subterminal, 390–625 (490)	subterminal, round 234–527 × 217–504 (364 × 359)
ventral sucker	round, muscular, 510–1035 (801)	round, slightly larger than oral sucker, 228–897 × 237–868 (502 × 489)
pharynx	elongate, ovoid, 125–232 × 94–215 (163 × 136)	ovoid, 65–153 × 65–153 (102 × 98)
oesophagus	18–42	74–322 × 15–134 (164 × 74)
caeca	intestinal bifurcation at anterior to ventral sucker; caeca terminate blindly from posterior extremity, 3000–4985 (3872) long	intestinal bifurcation at anterior to ventral sucker; caeca terminate near to posterior extremity, 1055–3709 × 50–254 (1896 × 109)
testes	two, symmetrical, spherical to ovoid. Left testis 250–432 × 209–382 (311 × 246) and right testis 249–382 × 197–309 (305 × 243)	two: left testis 49–276 × 33–262 (150 × 117) and right testis 45–261 × 31–294 (134 × 113)
cirrus-sac	large, elongate, post bifurcal, medially placed; seminal vesicle saccular, bipartite; 279–415 × 107–193 (366 × 159)	anterior to ventral sucker, post bifurcal, medially placed; containing bipartite seminal vesicle and ejaculatory duct; 70–191 × 37–107 (144 × 80)
ovary	posterior to ventral sucker, equatorial, round to ovoid 301–439 × 376–483 (345 × 406)	posterior to ventral sucker 66–218 × 61–235 (120 × 107)
vitellarium	single mass	single mass
uterus	fill whole post-testicular body, extends into entire extra-caecal space, up to the level of intestinal bifurcation	filled testicular region, extends extra-caecal up to the level of intestinal bifurcation
eggs	numerous, small, thin-shelled, non-operculate, embryonate, 78–86 × 70–83 (83 × 76)	numerous, round to oval, embryonate, 11–63 × 6–44 (34 × 22)

lateral fields or clearly identifiable vitellaria absent. Eggs small, round to oval, numerous, embryonated. Excretory vesicle I-shaped; pore terminal. In intestine of freshwater fishes. Type genus *Chelatrema* Gupta & Kumari, 1973.

Key to genera

1a. Oral sucker without lobes, smaller than ventral sucker; uterus intercaecal and extracaecal up to posterior extremity; sometimes extracaecal up to caecal bifurcation; vitellarium a single compact mass . . . . *Chelatrema* Gupta & Kumari, 1973

1b. Oral sucker with lobes, equal to or slightly larger than ventral sucker; uterus intercaecal up to posterior extremity; vitellarium variable, small follicles in lateral fields or lacking of clearly identifiable vitellaria . . . . *Paracreptatrematina* Amin & Myer, 1982

Discussion

The genus *Chelatrema* was described in the family Hemiuridae by Gupta & Kumari (1973). Gibson (2002) transferred it to family Gorgoderidae Looss, 1899. Later, Campbell (2008) stated that it should be considered as gorgoderid *genus inquirendum* pending for the study. The genus *Paracreptatrematina* Amin & Myer, 1982, from freshwater fish of the United States, was described in the family Allocreadiidae. According to Platta & Choudhury (2006) *P. limi* has unique oral muscular papillae (compared with other papillose allocreadiids) and absence of clearly identifiable vitellaria. Curran *et al.* (2011) concluded that *P. limi* does not belong to Allocreadiidae on the basis of molecular analysis. Members of the new family differ from each other mainly in the presence of a ventrolateral pair of triangular lobes in *P. limi* that are absent in *Chelatrema*. Curran *et al.* (2011) did not include *P. limi* under any named family nor its own family due to shortage of data related to the life history and genera that are closely related to this species.

Comparison of morphological characters of the proposed new family with those of other families under Gorgoderoidea as given by Bray & Blair (2008) showed some major difference in shape and position of testes and ovary, position of uterus, distribution of vitellarium and presence or absence of cirrus-sac. Members of the new family lack any papillae or spines on the tegument, which were reported in several members of Gorgoderidae, Brachycoeliidae Looss, 1899, Dicrocoeliidae, Mesocoeliidae, Paragonimidae, Prouterinidae and Troglotrematidae. The vitellarium in members of Dicrocoeliidae and Encyclometridae is usually limited in extent, forms two lateral bands or clusters, or rarely one band (Gupta & Mehrotra, 1977; Tkach *et al.*, 2018). Members of *Chelatrema* usually possess a single vitellarium, while *P. limi* has small follicles in lateral fields. Platta & Choudhury (2006) reported the absence of clearly identifiable vitellaria in *P. limi*.

In the present study, close phylogenetic relationships between *C. neilgherriensis* and *P. limi* have been revealed. Alongside with genetic-*P* distance analysis, these two species can be recognized as representatives of a new family within the Gorgoderoidea. *Paracreptatrematina limi* was described for the first time as a member of a new genus of Allocreadiidae from the mud-minnow *Uumbra limi* (Kirtland, 1840) from three states of the United States (Amin & Myer, 1982). The first representative taxonomic and phylogenetic studies on this species with a molecular tool were made by Curran *et al.* (2011), who showed an independent phylogenetic position of *P. limi* relative to Allocreadiidae and stated that this species was not an allocreadiid, but, probably, a member of its own family. Our results confirm this view, demonstrating *Chelatrema* from India as a species closely related to *P. limi*. Cases of close relationships of trematodes from Eurasian and North American continents have been observed for

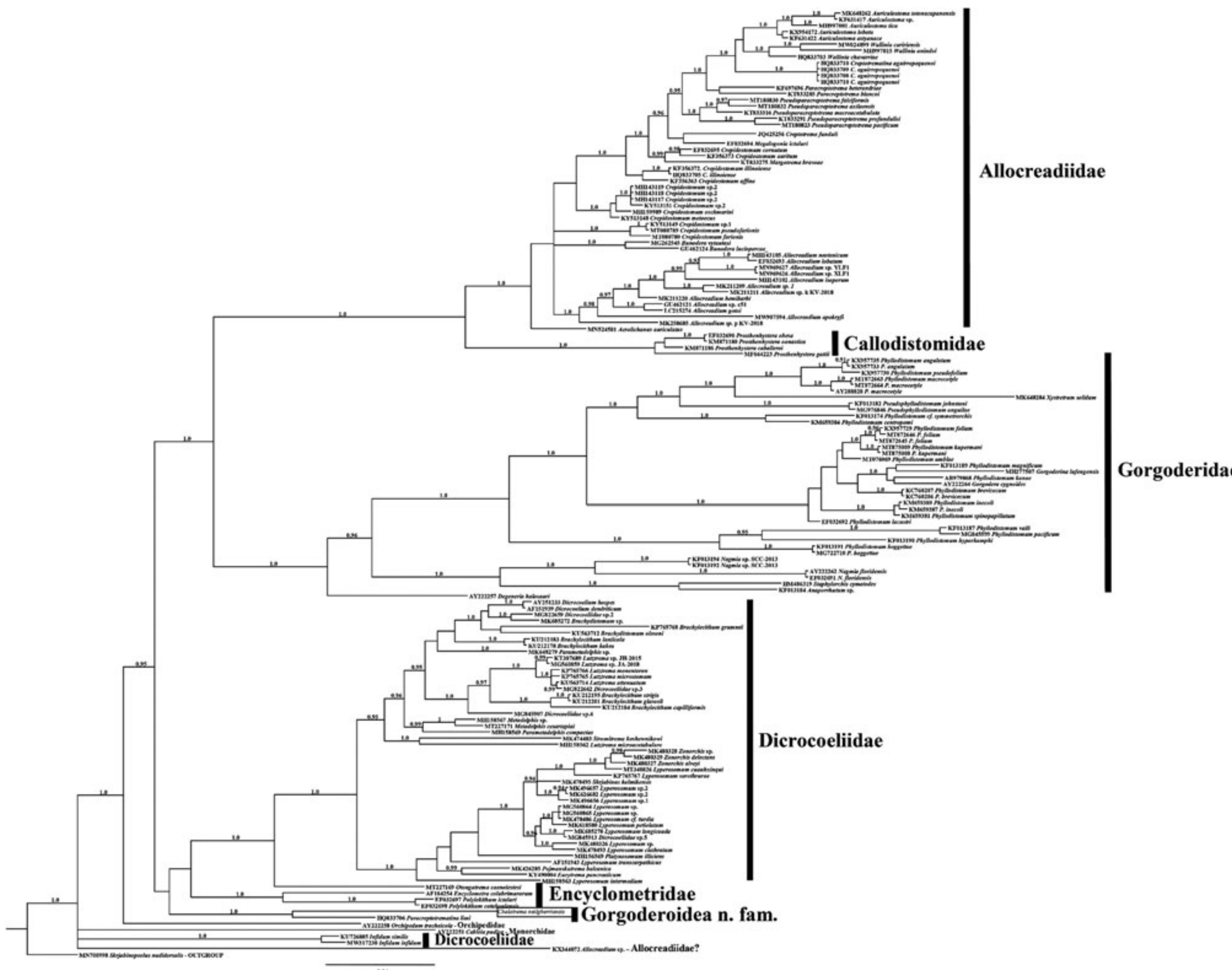

Fig. 2. Bayesian phylogenetic tree of Gorgoderoidea reconstructed on the basis of 28S rDNA sequences. Original sequences are in boldface type. Nodal numbers – *a posteriori* probability values (only significant values presented).

gorgoderoids. For example, detailed phylogenetic analysis of Allocreadiidae indicated such relationships, where North American species belong to a terminal clade, sister to Asian and European species within this family (Soldánová *et al.*, 2017; Petkevičiūtė *et al.*, 2018; Atopkin *et al.*, 2020; Faltýnkova *et al.*, 2020; Vainutis *et al.*, 2021; Bogatov & Vainutis, 2022). However, lack of molecular data on species, closely related to *C. neilgherriensis* and *P. limi* as well as for type-species of *Chelatrema* is a barrier to clarify these relationships. Moreover, accepting these two species as representatives of a separate new family, presence and origin of considerable morphological differences of its members should be further considered. Representative data on closely related trematodes for this new family are strongly needed to address these questions.

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Conflict of interests. None.

Ethical standards. All procedures performed in the study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted.

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