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Original Article

Catatropis indicus (Digenea: Notocotylidae) from ducks in Indonesia with a brief review of the genus *Catatropis*

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Abstract

Adult notocotylid flukes (Digenea: Notocotylidae) were recovered from the ceca of Pitalah ducks (*Anas* sp.) in Aceh Province, Indonesia. These flukes were morphologically characterized by a median ventral ridge with 2 lateral rows of ventral papillae and the absence of both a ventral sucker and pharynx, consistent with the characteristics of the genus *Catatropis*. They exhibited a genital pore located just posterior to the oral sucker, 10–11 pairs of ventral papillae, a deep and multi-lobed ovary and testes, a metraterm equal in length to the cirrus sac, and ceca bearing numerous diverticula; accordingly, they were identified as *Catatropis indicus* Srivastava, 1935. Adult specimens measured 3.01–3.77 mm (average 3.47 mm) in length and 0.98–1.21 mm (average 1.11 mm) in width (*n*=8). Uterine eggs measured 0.016–0.023 mm (average 0.019 mm) in length and 0.008–0.014 mm (average 0.012 mm) in width (*n*=20), each bearing 2 long polar filaments. These specimens resembled *Catatropis vietnamensis* Izrailskaia et al., 2019, and *Catatropis pakistanensis* Schuster and Wibbelt, 2012, sharing features such as a genital pore immediately posterior to the oral sucker and other morphological traits. However, they differed from *C. vietnamensis* by having a larger body, ceca with numerous diverticula, and a broader anterior distribution of vitelline follicles. They also differed from *C. pakistanensis* in possessing a longer esophagus and ceca with multiple diverticula. In 28S rDNA sequence analysis, our specimens showed 99.1% identity with both *C. indicus* and *C. vietnamensis*. In contrast, internal transcribed spacer 2 (ITS2) sequence comparisons revealed only 96.0%–96.1% identity with *C. vietnamensis* (no GenBank data available for *C. indicus*), suggesting that our specimens are phylogenetically distant from *C. vietnamensis*. This represents the first report of *C. indicus* from ducks in Indonesia. A brief review of *Catatropis* species is provided.

Keywords: *Catatropis indicus*, duck (*Anas* sp.), intestinal fluke, molecular analysis, Indonesia

Introduction

The genera *Catatropis* Odhner, 1905, and *Notocotylus* Diesing, 1839 (Digenea: Notocotylidae), are trematodes that parasitize the intestines of birds and, less commonly, mammals [1-3]. Infected hosts may develop severe conditions such as diarrhea, anemia, malabsorption, weight loss, general debility, or even death [2]. These genera are morphologically characterized by longitudinal ridges or papillae on the ventral surface, eggs bearing long bipolar filaments, and the absence of both a ventral sucker and pharynx [1-3]. The primary

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Data curation: Chai JY, Jung BK, Chang T, Hong S, Shin H
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Validation: Chai JY, Jung BK
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Conflict of interest
The authors declare no conflict of interest related to this study.

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distinction lies in body structure: *Catatropis* species possess a median ventral ridge flanked by 2 separate rows of ventral papillae, whereas *Notocolytus* species exhibit 3 rows of ventral papillae without a median ridge [1-3].

Within the genus *Catatropis*, at least 30 species have been described (Table 1) [2-31]. Of these, 23 are listed in WoRMS (https://www.marinespecies.org), while 7 are excluded. Two were omitted due to taxonomic ambiguity (*Catatropis orientalis* Harshe, 1932 [4]) or the lack of deposited type material (*Catatropis vietnamensis* Izrailskaia, Besprozvannykh, Tatanova, Nguyen, and Ngo, 2019 [3]). *Catatropis filamentis* Barker, 1911 [5], and *Catatropis pacifera* Noble, 1933 [6], were excluded by Bayssade-Dufour et al. [7] and Schuster and Wibbelt [8] because of structural anomalies in the median ridge. *Catatropis joyeuxi* Dvorjadkin, 1989 [9], was excluded due to the absence of type material [7]. However, the rationale behind the exclusion of *Catatropis aegyptiacus* Ramadan, Abdou, Taha, and Haroun, 2020 [10], and *Catatropis pakistanensis* Shafi, Rehana, and Samad, 1982 (from mammalian host) [11], remains unclear.

We tentatively included *C. aegyptiacus*, *C. orientalis*, *C. pakistanensis* (from a mammalian host), and *C. vietnamensis* in the list of *Catatropis* species, while excluding 5 species listed in WoRMS: *Catatropis appendiculata* Lutz, 1928 [12]; *Catatropis charadrii* Skrjabin, 1915 [13]; *Catatropis gallinulae* Johnston, 1928 [14]; *Catatropis johnstoni* Martin, 1956 [15]; and *Catatropis nicolli* Cribb, 1991 [16]. *C. appendiculata* was not included by McDonald in 1981 [17] due to the absence of distinguishing features beyond the presence of numerous short diverticula along the intestinal ceca. *C. charadrii* was synonymized with *C. verrucosa* Odhner, 1905, by Kanev in 1994 based on close morphological similarity [18]. *C. gallinulae* was excluded because of an improperly structured median ridge [7,8]. *C. johnstoni* and *C. nicolli* were omitted due to the absence of bilateral rows of ventral papillae [7]. As a result, a total of 22 species are considered tentatively valid in this study (Tables 1, 2) [2-31].

Catatropis verrucosa [synonyms *Fasciola verrucosa* Frölich, 1789; *Fasciola anseris* Gmelin, 1790; *Monostoma verrucosa* (Frölich, 1789) Zedar, 1800; *C. charadrii* Skrjabin, 1915] was originally described from wild geese (*Anser anser*) in Germany [19] and later redescribed by Kanev et al. [18]. *Catatropis indicus* Srivastava, 1935, was identified based on adult flukes recovered from the rectal ceca of the Indian fowl (*Gallus bankiva murghi*) in India [2]. *C. aegyptiacus* was recovered from Norway rats (*Rattus norvegicus*) in Egypt [10]. *C. chilinae* Flores and Brugni, 2003, was found in chickens and ducks experimentally infected with metacercariae encysted in the environment near the freshwater snail (*Chilina dombeiana*) in Argentina [20]. *C. chinensis* Lai, Sha, Zhang, and Yang, 1984, was collected from chickens and ducks in China [21]. *C. cygni* Yamaguti, 1939, was reported from the bird (*Cygnus bewickii jankowskii*) in Japan [22]. *C. harwoodi* Bullock, 1952, was discovered in the Canadian goose (*Branta canadensis*) in the USA [23]. *C. hatcheri* Flores and Brugni, 2006, was described from chickens and ducks experimentally infected with metacercariae encysted near the freshwater snail (*Heleobia hatcheri*) in Argentina [24]. *Catatropis hisikui* Yamaguti, 1939, was reported from the bird (*Anser fabalis serrirostris*) in Japan [22]. *Catatropis kashimirensis* Khan and Chishti, 1987, was collected from chickens (*Gallus domesticus*) in India [25]. *Catatropis lagunae* Bayssade-Dufour, Albaret, Fermet-Quinet, and Farhati, 1996, was found in experimental birds (*Anas anser*, *Anas platyrhynchos*, and *Cairina moschata*) infected with metacercariae in France [7]. *C. liara* Kossak, 1911 (syn. *Monosto-*

Table 1. Thirty *Catatropis* spp. reported in the literature

Species name/nominator & year [reference]	WoRMS (marinespecies.org)	Schuster and Wibblet [8]	Tentatively valid species (this study)	Remarks
C. appendiculata Lutz, 1928 [12]	Listed	-	-	Not acknowledged as a distinct species (McDonald [17])
C. aegyptiacus Ramadan et al., 2020 [10]	-	-	Listed	-
C. charadrii Skrjabin, 1915 [13]	Listed	-	-	Synonymized with *C. verrucosa* (Kanev et al. [18])
C. chilinae Flores and Brugni, 2003 [20]	Listed	Listed	Listed	-
C. chinensis Lai et al., 1984 [21]	Listed	Listed	Listed	-
C. cygni Yamaguti, 1939 [22]	Listed	Listed	Listed	-
C. filamentis Barker, 1911 [5]	-	-	-	Removed from this genus (Bayssade-Dufour et al. [7])
C. gallinulae Johnston, 1928 [14]	Listed	-	-	Removed from this genus (Bayssade-Dufour et al. [7])
C. harwoodi Bullock, 1952 [23]	Listed	Listed	Listed	-
C. hatcheri Flores and Brugni, 2006 [24]	Listed	Listed	Listed	-
C. hisikui Yamaguti, 1939 [22]	Listed	Listed	Listed	-
C. indicus Srivastava, 1935 [2]	Listed	Listed	Listed	-
C. johnstoni Martin, 1956 [15]	Listed	-	-	Removed from this genus (Bayssade-Dufour et al. [7])
C. joyeuxi Dvorjadkin, 1989 [9]	-	-	-	No deposition of type material (Schuster and Wibbelt [8])
C. kashmirensis Khan and Chishti, 1987 [25]	Listed	-	Listed	-
C. lagunae Bayssade-Dufour et al., 1996 [7]	Listed	Listed	Listed	-
C. liara Kossak, 1911 [26]	Listed	Listed	Listed	-
C. misrai Gupta and Singh, 1984 [27]	Listed	Listed	Listed	-
C. morosovi Gubanow et al., 1966 [28]	Listed	Listed	Listed	-
C. onobae Gonchar and Galaktionov, 2021 [29]	Listed	-	Listed	-
C. orientalis Harshe, 1932 [4]	-	Listed	Listed	Not listed by WoRMS (uncertain taxon)
C. nicolli Cribb, 1991 [16]	Listed	-	-	Removed from this genus (Bayssade-Dufour et al. [7])
C. pacifera Noble, 1933 [6]	-	-	-	Removed from this genus (Bayssade-Dufour et al. [7])
C. pakistanensis Shafi et al., 1982 [11]	-	Listed	Listed	-
C. pakistanensis[a] Schuster and Wibbelt, 2012 [8]	Listed	-	Listed	-
C. poecilorhynchai Gupta and Singh, 1984 [27]	Listed	Listed	Listed	-
C. pricei Harwood, 1939 [30]	Listed	Listed	Listed	-
C. rauschi Sing, 1956 [31]	Listed	Listed	Listed	-
C. verrucosa Odhner, 1905 [19]	Listed	Listed	Listed	-
C. vietnamensis Izrailskaia et al., 2019 [3]	-	-	Listed	Not listed by WoRMS (no deposition of type material)

[a]This name was reported as duplicate, unaware of the previous nomenclature by Shafi et al. [11]. This species should be renamed.

mum attenuatum Lühe, 1898), was redescribed based on specimens from the bird (*Phenicopterus roscus*) in Tunisia [26]. *C. misrai* Gupta and Singh, 1984, was described from Indian birds (*Sarkidiornis melanotos* and *Gallinula chloropus*) [27]. *C. morosovi* Gubanov, Fedorov, Berlovskaya, and Kusnetsova, 1966, was identified from the water vole (*Arvicola terrestris*) in Russia [28]. *C. onobae* Gonchar and Galaktionov, 2021, was found in birds (*Somateria mollissima*) as adult flukes and in snails (*Onoba aculeus*) as rediae and cercariae in Russia and Iceland [29]. *C. orientalis* Harshe, 1932, was reported from wild ducks (*Dafila acuta acuta*) in India [4]. *C. pakistanensis* Shafi, Rehana, and Samad, 1982,

Table 2. Twenty-two tentatively valid *Catatropis* spp. included in this study

Species	Host	Position of genital pore	No. of ventral papillae	Country of original report
C. aegyptiacus	Mammal	Anterior to ceca bifurcation	10–11 pairs	Egypt
C. chilinae	Bird	Posterior to ceca bifurcation	9–11 pairs	Argentina
C. chinensis	Bird	Anterior to ceca bifurcation	12 pairs	China
C. cygni	Bird	Posterior to ceca bifurcation	12–18 pairs	Japan
C. harwoodi	Bird	Anterior to ceca bifurcation	7–9 pairs	USA
C. hatcheri	Bird	Posterior to ceca bifurcation	10–12 pairs	Argentina
C. hisikui	Bird	Posterior to ceca bifurcation	15–17 pairs	Japan
C. indicus	Bird	Anterior to ceca bifurcation	10–13 pairs	India
C. kashmirensis	Bird	Near ceca bifurcation	9 pairs	India
C. lagunae	Bird	Posterior to ceca bifurcation	6–9 pairs	France
C. liara	Bird	Posterior to ceca bifurcation	>12 pairs	Tunisia
C. misrai	Bird	Anterior to ceca bifurcation	12 pairs	India
C. morosovi	Mammal	Posterior to ceca bifurcation	13 pairs	Russia
C. onobae	Bird	Near ceca bifurcation (3 areas)	8–12 pairs	Russia and Iceland
C. orientalis	Bird	Posterior to ceca bifurcation	7–8 pairs	India
C. pakistanensis (mammal)	Mammal	Posterior to ceca bifurcation	12 pairs	Pakistan
C. pakistanensis (bird)	Bird	Anterior to ceca bifurcation	9–10 pairs	Pakistan
C. poecilorhynchai	Bird	Anterior to ceca bifurcation	4–6 pairs	India
C. pricei	Bird	Anterior to ceca bifurcation	9–11 pairs	USA
C. rauschi	Bird	Anterior to ceca bifurcation	10 pairs	India
C. verrucosa	Bird	Posterior to ceca bifurcation	8–12 pairs+1 (center)	Germany
C. vietnamensis	Bird	Anterior to ceca bifurcation	9 pairs	Vietnam

was recovered from rice rats (*Bandicota bengalensis*) in Pakistan [11]. *C. pakistanensis* Schuster and Wibbelt, 2012, was identified from Northern shoveler ducks (*Anas clypeata*) in Pakistan [8]. *C. poecilorhynchai* Gupta and Singh, 1984 (syn. *C. rauschi* of Gupta and Jahan, 1977), was found in Indian birds (*Anas poecilorhyncha*) [27]. *C. pricei* Harwood, 1939, was described based on specimens from the Canadian goose (*Branta canadensis*) in the USA [30]. *C. rauschi* Sing, 1956, was identified from the pintail duck (*Dafila acuta*) in India [31]. *C. vietnamensis* Izrailskaia et al., 2019, was collected from experimental ducks (*Anas platyrhynchos*) infected with metacercariae encysted near the snail (*Melanoides tuberculata*) in Vietnam [3].

We surveyed intestinal flukes infecting ducks in Aceh Province, Indonesia, and identified adult flukes belonging to the family Notocotylidae. These were characterized by a median ventral ridge flanked by a pair of ventral papillae on each side and by the absence of a ventral sucker and pharynx. Based on these features, we classified our specimens in the genus *Catatropis*. Detailed morphological examination led to their identification as *C. indicus*. Molecular analyses of the 28S rDNA and ITS2 genes further supported their placement within the genus *Catatropis*.

The pathogenicity of *C. indicus* is high, as infected birds were found severely ill or dead, displaying pronounced signs such as emaciation, weight loss, anemia, dull plumage, and poor overall condition [2]. The life cycle of this species was previously studied in Malaysia, where *Bithynia* sp. snails served as intermediate hosts. Metacercariae were observed encysted on snail shells, aquatic vegetation, and the surfaces of glass containers, while adult

flukes were recovered from experimentally infected chickens [32]. Additional studies have described the nervous system of the cercariae [33] and the ultrastructure of the tegumental surface of adult flukes [34]. The presence of *C. indicus* has been confirmed in Australia [35], Egypt [36], and Indonesia (this study), but not in other countries.

The present report aims to describe the morphological features of *C. indicus* collected from the duck intestines in Indonesia. A brief review is also provided on the 22 tentatively valid *Catatropis* spp. reported in the literature.

Materials and Methods

Ethics statement

All animal experiments were conducted in accordance with the ARRIVE guidelines for animal research (https://arriveguidelines.org) and were approved by the Institutional Animal Care and Use Committee of the MediCheck Research Institute, Korea Association of Health Promotion, Seoul, Korea (KAHP 201805).

Collection of helminth specimens

Pitalah ducks (*Anas* sp.) were collected in Aceh Province, Indonesia, between March and October 2018. A total of 20 ducks were examined, sourced from 2 counties: Aceh Besar and Matang Glumpang. The ducks were euthanized via cervical dislocation, and the entire intestinal tract, including both the small and large intestines, was resected. The intestines were opened longitudinally using scissors and examined under a stereomicroscope for helminth parasites, including trematodes. For morphological analysis, trematode specimens were fixed in 10% formalin on a glass slide and covered with a cover slip. Some specimens were preserved in 70%–90% ethanol for molecular studies.

Morphological study

Formalin-fixed specimens were thoroughly rinsed in water and stained with Semichon's acetocarmine. They were subsequently dehydrated in a graded ethanol series (70%–100%), cleared in xylene, and mounted on glass slides using Permount (Fisher Scientific, Pittsburgh, PA, USA). The specimens were examined morphologically under a light microscope (CX33, Olympus, Tokyo, Japan). All measurements are expressed in millimeters, unless otherwise specified.

Molecular and phylogenetic analyses

For molecular analyses, sequences of nuclear genes, including 28S ribosomal DNA (28S rDNA) and internal transcribed spacer 2 (ITS2), were obtained. Genomic DNA from each specimen was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. PCR amplification and sequencing were conducted using specific primers: dig12 and LO for 28S rDNA and BR and dig11 for ITS2, under conditions based on previously published protocols [37,38]. Multiple sequences were aligned using Clustal W software 2.1 (University College Dublin, Dublin, Ireland) [39]. A phylogenetic tree, incorporating isolates obtained in this study and reference sequences of trematodes such as *Catatropis* sp. and *Notocotylus* sp. from the GenBank database, was

constructed using the maximum-likelihood method with the Tamura-Nei model [40] for nucleotide substitution. The tree was visualized using MEGA v6 software [41], and bootstrap values were calculated based on 1,000 replications.

Results

Helminth species collected

Two species of trematodes and one unidentified species of cestode were recovered from the intestinal tract of the ducks. One trematode species, isolated from the small intestine, was identified as *Echinostoma miyagawai*, previously reported by Chai et al. [42]. The second trematode collected from the large intestine (ceca) was identified as *C. indicus*, as confirmed in this study. Specimens of *C. indicus* were found in 5 of the 20 ducks (25.0%), with a total of 48 specimens recovered, averaging 9.6 specimens per infected duck.

Brief morphological descriptions

Family Notocotylidae Lühe, 1909

Genus *Catatropis* Odhner, 1905

Species *C. indicus* Srivastava, 1935 (Table 3; Fig. 1A-E) [2,3,32]

Description (adults, *n*=8): The body is elongated, flat, and semi-transparent, ventrally concave, tapering anteriorly, and rounded posteriorly, measuring 3.01–3.77 mm in length (average 3.47 mm) and 0.98–1.21 mm in width (average 1.11 mm). The tegument is covered with minute, scale-like spines, which become sparse toward the posterior end. A ven-

Table 3. Morphology and measurements (mm) of *Catatropis indicus* adults compared with previous reports and a closely related species

Characters	*C. indicus* This study (*n*=8)	*C. indicus* Srivastava (1935) [2] (*n*=?)	*C. indicus* Rohde and Lee (1967) [32] (*n*=20)	*C. vietnamensis* Izrailskaia et al. (2019) [3] (*n*=10)
Body (L×W)	3.01–3.77×0.98–1.21	4.20–4.60×1.20	2.22–4.34×0.83–1.17	1.46–1.60×0.52–0.55
Oral sucker (L×W)	0.14–0.17×0.11–0.16	0.14–0.20 (diam.)	0.13–0.17×0.14–0.21	0.09×0.11–0.12
Esophagus (L)	0.14–0.25	0.20–0.26	-	0.10–0.12
Cirrus sac (L×W)	0.98–1.20×0.15–0.22	0.87–1.20×0.17–0.20	0.91–1.13×0.14–0.21	0.51–0.60×0.05–0.08
Testis, left (L×W)	0.54–0.75×0.26–0.33	0.75–0.99×0.20–0.30	0.50–0.86×0.20–0.35	0.21–0.28×0.17–0.18
Testis, right (L×W)	0.59–0.78×0.27–0.35	0.75–0.99×0.20–0.30	0.50–0.86×0.20–0.35	0.27–0.29×0.15–0.17
Ovary (L×W)	0.24–0.35×0.20–0.26	0.26–0.35×0.26–0.35	0.18–0.40×0.15–0.29	0.12–0.16×0.12
No. of ovarian lobes	8–10 lobes	Irregularly lobed	Slightly lobed	4 lobes
Mehlis' gland (L×W)	0.20–0.25×0.15–0.23	0.23–0.26×0.17–0.26	-	0.06–0.08×0.09–0.10
No. of uterine loops	16–18	18	17–18	15–18
Genital pore (position)	Immediately behind oral sucker	Immediately behind oral sucker	Immediately behind oral sucker	Immediately behind oral sucker
Metraterm (L)	0.90–1.20	0.87–1.20	0.91–1.13	0.18–0.19
Vitelline fields (L)	0.87–1.70	1.20–1.50	0.61–1.33	0.27–0.39
No. of ventral papillae (glands) on each side	10–11	10–12	12–13	9
Eggs (L×W)	0.016–0.023×0.008–0.014	0.0170–0.020×0.008–0.010	0.022–0.027×0.009–0.011	0.019–0.023×0.008–0.012
Length of filaments	0.140–0.180	-	0.150–0.230	-
Ceca diverticula	Numerous	Numerous	Numerous	Almost no

L, length; W, width.

tral ridge extends longitudinally from the level of the cirrus sac to the posterior margin of the ovary. Two lateral rows of ventral papillae (10–11 per side) flank the ventral ridge. The first pair of papillae is located near the 15th–16th uterine coils, posterior to the cirrus sac, while the last pair is situated at the level of the posterior two-thirds of the testes. The oral sucker is round and subterminal, with no pharynx or ventral sucker. The short esophagus bifurcates into 2 ceca, which undulate markedly and form numerous fine diverticula projecting both inward and outward. The ceca extend laterally around the uterine coils and terminate near the posterior margin of the testes. The testes are symmetrical, deeply lobed, and located near the posterior extremity of the body. The external seminal vesicle is round or coiled, with an elongated posterior segment that connects to the internal seminal vesicle. The cirrus sac is elongated, tapering anteriorly and broadening posteriorly, and houses the internal seminal vesicle, prostatic gland, and unarmed cirrus. The genital pore is located just posterior to the oral sucker and contains male and female openings; the female pore leads to the metraterm and subsequently to the uterine coils. The metraterm extends approximately two-thirds the length of the cirrus sac. The uterine coil runs from the anterior margin of Mehlis' gland to the posterior level of the cirrus sac, forming 16–18 coils. Mehlis' gland varies in shape, most commonly triangular or quadrangular, and is located anterior

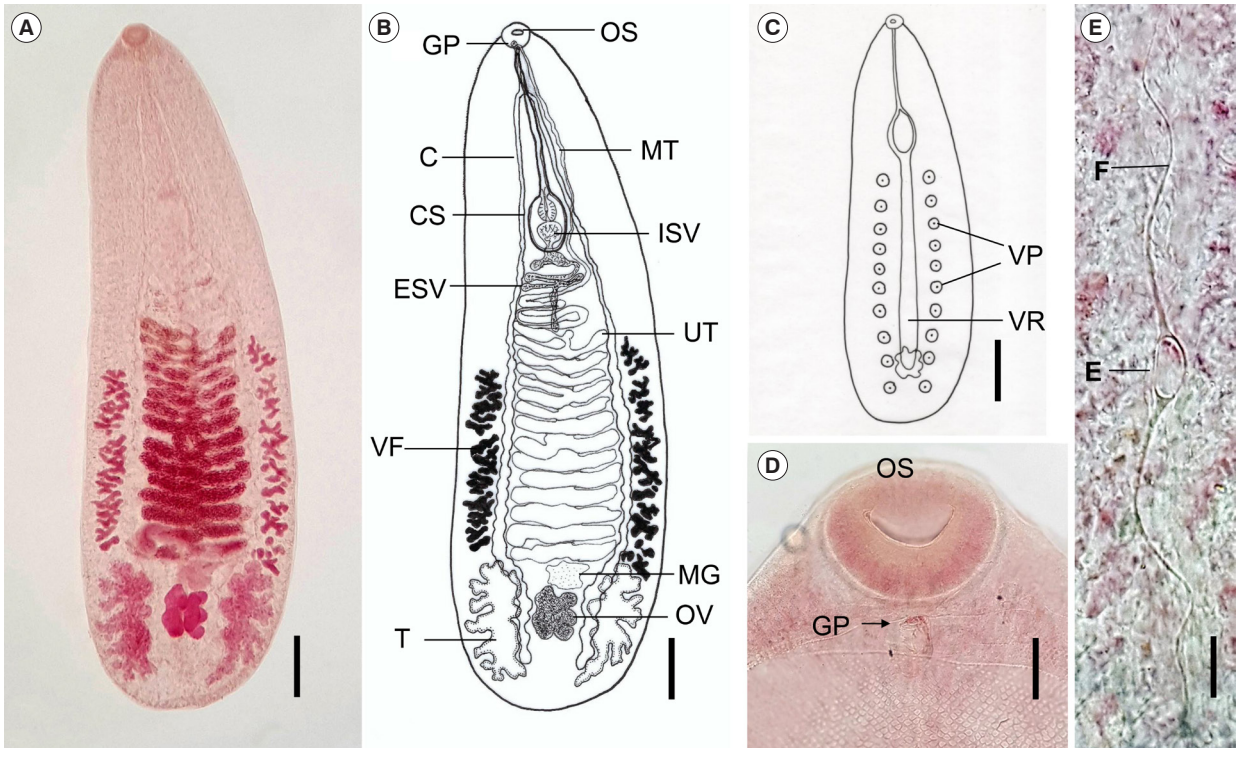

Fig. 1. *Catatropis indicus* (adult) collected from ducks (*Anas* sp.) in Aceh Province, Indonesia. (A) Adult fluke stained with acetocarmine, showing the oral sucker, uterine coils, vitelline follicles, Mehlis' gland, ovary, and testes. Ventral view. Scale bar=0.3 mm. (B) Line drawing of the adult fluke shown in (A). Scale bar=0.3 mm. (C) Illustration showing the median ventral ridge (VR) and 10 ventral papillae (VP) on each side. Scale bar=0.5 mm. (D) Location of the genital pore (GP) immediately posterior to the oral sucker (OS). Scale bar=0.05 mm. (E) Egg (E) with a pair of polar filaments (F) in the distal uterine coil. Scale bar=0.018 mm. C, cecum; CS, cirrus sac; ESV, external seminal vesicle; VF, vitelline follicles; T, testis; MT, metraterm; ISV, internal seminal vesicle; UT, uterus; MG, Mehlis' gland; OV, ovary.

to the ovary. The ovary is median, positioned between the testes, and extensively lobed into 8–10 lobes. The vitellaria consist of 2 lateral rows of irregularly shaped follicles, located outside the ceca, extending from the anterior edge of Mehlis' gland to the level of the 12th–13th uterine coils. The eggs are small, oval, and operculate, measuring 0.016–0.023 mm in length (average 0.019 mm) and 0.008–0.014 mm in width (average 0.012 mm), each bearing 2 polar filaments. The excretory vesicle is sac-like and possesses diverticula.

Taxonomic summary

Host: Pitalah duck (*Anas* sp.)

Site of infection: large intestine (cecum)

Locality: Aceh Province, Indonesia

Deposition of specimens: Parasite Museum, MediCheck Research Institute, Korea Asso-

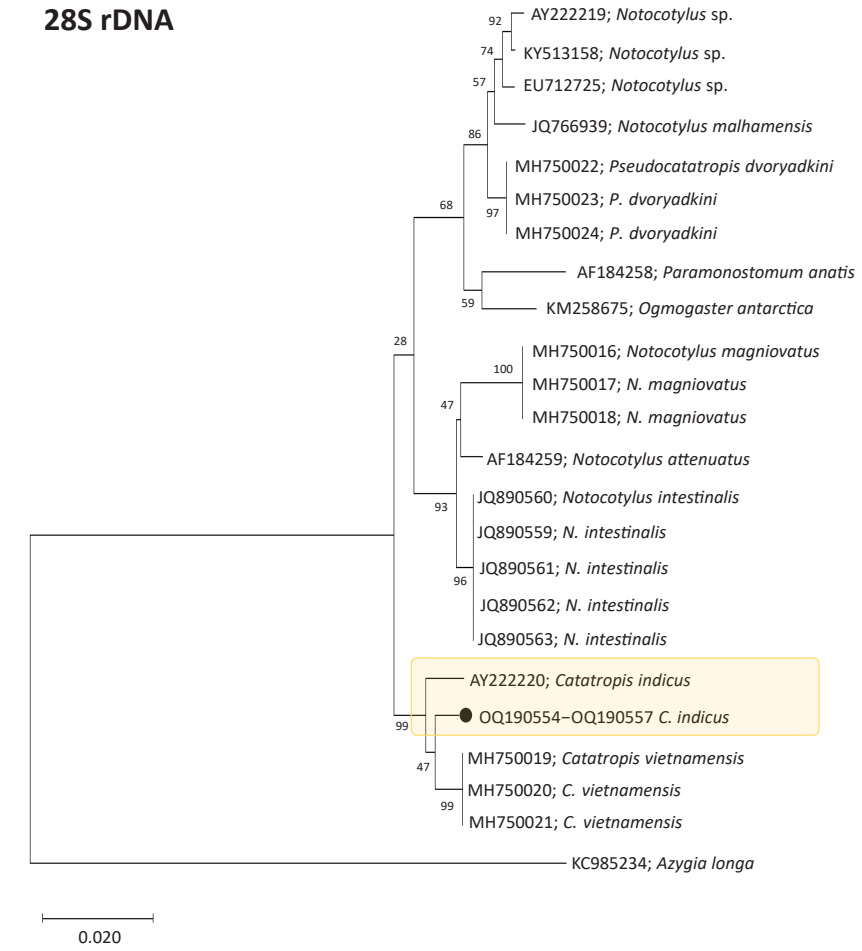

Fig. 2. Phylogenetic tree showing *Catatropis indicus* (*n*=4, GenBank No. OQ190554-OQ190557) and related species of *Catatropis*, *Notocotylus*, and *Pseudocatatropis* based on 28S rDNA sequences. *Azygia longa* served as the outgroup. Numeric values on the branches indicate support for the nodes generated by the maximum likelihood method using the Tamura-Nei model for nucleotide substitution. The tree was visualized using MEGA v6 software, and bootstrap values were obtained from 1,000 replicates. (●) indicates the specimens obtained in this study.

ciation of Health Promotion, Seoul 07649, Korea (No. 2024-0001-01~08). Two additional specimens are deposited in the Meguro Parasitological Museum, Tokyo 153-0064, Japan (MPM Coll. No. 25267).

Molecular results and phylogenetic trees

Partial sequences of the 28S rDNA (605 bp) (n=4, GenBank No. OQ190554–OQ190557) and ITS2 genes (257 bp) (n=4, GenBank No. OQ190558–OQ190561) from adult flukes were phylogenetically grouped in *Catatropis* spp. of the family Notocotylidae (Figs. 2, 3). The phylogenetic tree based on 28S rDNA showed that our samples clustered closely with a clade containing *C. indicus* and *C. vietnamensis*, both with 99.1% sequence identity (Fig. 2). However, the ITS2-based tree indicated that our samples formed a distinct lineage, separate from other members of the Notocotylidae, including *C. vietnamensis* (96.0%–96.1%),

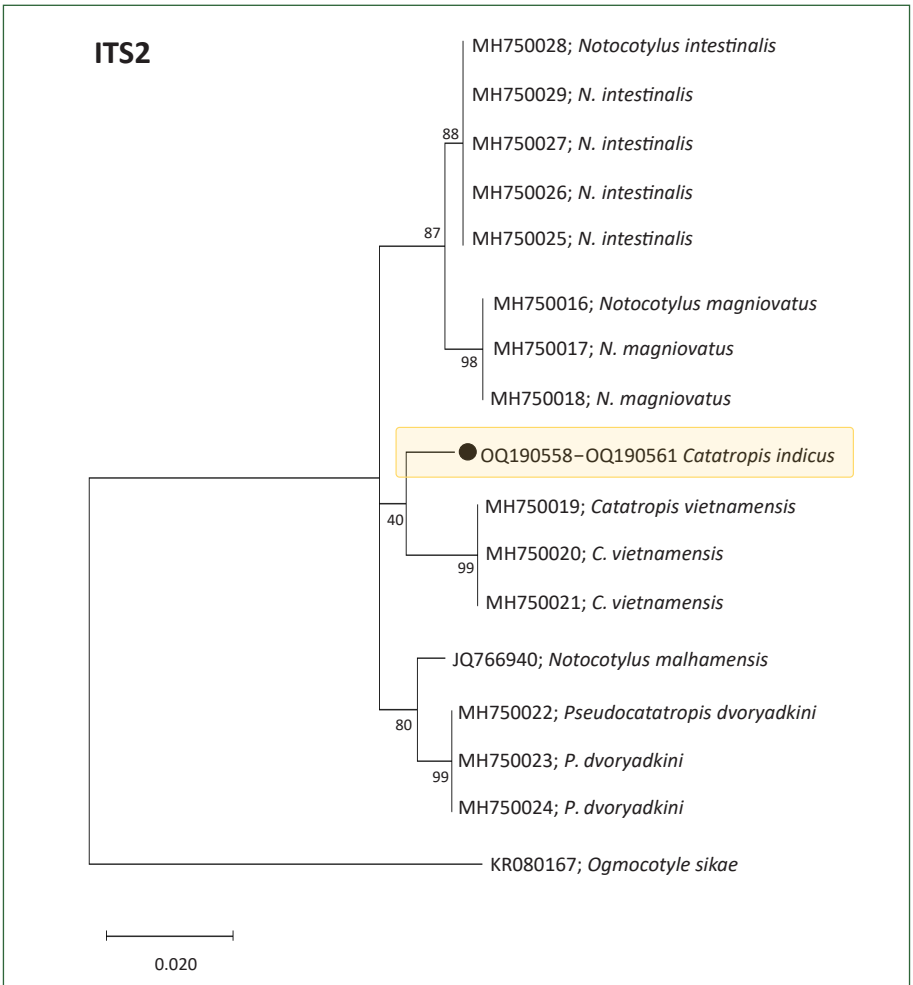

Fig. 3. Phylogenetic tree illustrating *Catatropis indicus* (n=4, GenBank No. OQ190558–OQ190561) and related species of *Catatropis*, *Notocotylus*, and *Pseudocatatropis* based on ITS2 sequences. *Ogmocotyle sikae* was used as the outgroup. Numeric values at the branches represent node support based on the maximum likelihood method under the Tamura-Nei model. The tree was visualized using MEGA v6 software, and bootstrap values were obtained from 1,000 replicates. (●) indicates the specimens obtained in this study.

Notocotylus magniovatus (95.3%), *N. intestinalis* (95.9%–96.1%), and *Pseudocatatropis dvoryadkini* (95.7%) (Fig. 3).

Discussion

Flukes of the family Notocotylidae Lühe, 1909, are parasites that primarily infect digestive tracts, particularly the ceca, of birds and mammals [43]. They are small, monostomatous (lacking a ventral sucker), dorsoventrally flattened, and generally appear elongated or oval. A distinctive feature of this family is the presence of papillae and/or ridges on the ventral surface. The arrangement of these structures serves as a key diagnostic criterion for differentiating genera, including *Hippocrepis* Travassos, 1922; *Notocotyloides* Dollfus, 1966; *Parapronocephalum* Belopol'skaya, 1952; *Ogmocotyle* Skrjabin & Schulz, 1933; *Ogmogaster* Jägerskiöld, 1891; *Pseudoquinqueserialis* Sutton, 1981; *Paramonostomum* Lühe, 1909; *Tristriata* Belopol'skaya in Skrjabin, 1953; *Catatropis* Odhner, 1905; *Notocotylus* Diesing, 1839; *Uniserialis* Beverley-Burton, 1958; *Tetraserialis* Petrov & Chertkova, 1960; and *Quinqueserialis* Skvortsov, 1935 [43]. The proposed functions of the papillae and ridges include facilitating host attachment, secreting digestive enzymes, and nutrient absorption; however, ultrastructural studies indicate that their precise roles remain unclear and warrant further investigation [43].

Within the genus *Catatropis*, 22 species are considered tentatively valid. However, taxonomic ambiguities persist. One such issue involves *C. pakistanensis*, which has been reported under the same name on 2 separate occasions (Tables 1, 2) [2-31]. The first report, published in 1982, described specimens collected from the stomach of the rice rat (*Bandicota bengalensis*) in Pakistan [11]. Unaware of this earlier report, Schuster and Wibbelt [8] later described a different species as *C. pakistanensis* based on specimens from the ceca of shoveler ducks (*Anas clypeata*), also in Pakistan. Therefore, the *C. pakistanensis* specimens from shovelers should be renamed. In addition to this nomenclatural conflict, other taxonomic debates may also arise regarding *Catatropis* species. There is an urgent need for expanded molecular data to clarify the taxonomy and evolutionary relationships within this genus.

Our specimens exhibited a median ventral ridge with a pair of ventral papillae on each side, consistent with diagnostic features of the genus *Catatropis*. Additional distinguishing characteristics included a genital pore located just posterior to the oral sucker, 10–11 ventral papillae per row, an ovary with 8-10 lobes, and ceca densely filled with numerous fine diverticula. Of the 22 tentatively recognized *Catatropis* species, 10 are reported to possess the genital pore anterior to the cecal bifurcation: *C. aegyptiacus* [10], *C. chinensis* [21], *C. harwoodi* [23], *C. indicus* [2], *C. misrai* [27], *C. pakistanensis* (from birds) [8], *C. poecilorhynchai* [27], *C. pricei* [30], *C. rauschi* [31], and *C. vietnamensis* [3] (Table 2). Our specimens aligned closely with previously published descriptions of *C. indicus*. In terms of ventral papillae, our specimens had 10–11 pairs per row. The original description of *C. indicus* reported 10–12 ventral papillae [2], while subsequent redescriptions noted 12–13 papillae [32,34,35]. Among the 22 tentatively valid *Catatropis* species, similar counts of ventral papillae were observed in *C. cygni* (12–18 pairs), *C. onobae* (8–12), and *C. verrucosa* (8–12). However, these species differ from our specimens in the position of the genital pore; it is

located posterior to the cecal bifurcation in *C. cygni* and *C. verrucosa* and near the bifurcation in *C. onobae* (Table 2).

Our specimens can also be distinguished from *C. aegyptiacus*, which produces notably larger eggs and has been reported in mammalian hosts, particularly rats [10]. *C. chinensis* differs in having less branched testes and a more simply lobed ovary [21]. *C. harwoodi* is characterized by a genital pore positioned midway between the oral sucker and cecal bifurcation, along with a metraterm that exceeds the length of the cirrus sac [17,23]. *C. misrai* is distinct in having an unspined body and a more restricted distribution of vitelline follicles [27]. *C. pakistanensis* (from birds) is characterized by a very short esophagus and ceca that lack prominent diverticula [8]. *C. poecilorhynchai* is easily identified by having only 4–6 pairs of ventral papillae [27,44]. *C. pricei* is distinguished by possessing separate genital openings, male (located caudal to the oral sucker) and female (positioned mid-esophagus), along with a less lobulated ovary and testes [17,30]. *C. rauschi* can be differentiated by a metraterm that is longer than the cirrus sac [31]. *C. vietnamensis* differs in having a smaller body size, a less lobed ovary, a shorter metraterm, fewer vitelline follicles, and ceca that nearly lack diverticula [3].

In molecular analyses, the 28S rDNA sequences of our specimens (GenBank No. OQ190554–OQ190557, Indonesia) showed a high identity of 99.1% with both *C. indicus* (from Australia) and *C. vietnamensis* (from Vietnam). However, for ITS2 sequences, no reference data for *C. indicus* were available in GenBank. The highest identity of our specimens based on ITS2 sequences was 96.0%–96.1% with *C. vietnamensis* (Vietnam), followed by 95.9%–96.1% with *N. intestinalis* (Vietnam). This result prevents conclusive species-level identification. These molecular findings support the classification of our specimens within the genus *Catatropis*, but not definitely as *C. indicus*. Additional molecular data are needed to resolve this taxonomic uncertainty.

The differential diagnosis of species within the genus *Catatropis* can be conducted as follows (updated and modified from McDonald [17]):

1. Parasites of mammals ---------- 2
   Parasites of birds ---------- 4
2. Genital pore anterior to cecal bifurcation, ventral papillae 10–11 pairs -- *C. aegyptiacus*
   Genital pore posterior to cecal bifurcation, ventral papillae ≥12 pairs ---------- 3
3. Ventral papillae: 12 pairs; vitellaria start far below cirrus sac ---------- *C. morosovi*
   Ventral papillae: 13 pairs; vitellaria start just below cirrus sac ------ *C. pakistanensis* (rat)
4. Genital pore near cecal bifurcation ---------- 5
   Genital pore anterior to cecal bifurcation ---------- 6
   Genital pore posterior to cecal bifurcation ---------- 10
5. Ventral papillae: 8–12 pairs, unequal in size; uterus 14–21 coils ---------- *C. onobae*
   Ventral papillae: 9 pairs, equal in size; uterus 17 coils ---------- *C. kashimirensis*
6. Ventral papillae: 4–6 pairs ---------- *C. poecilorhynchai*
   Ventral papillae: 7–9 pairs ---------- 7
   Ventral papillae: 9 or 10 pairs ---------- 8
   Ventral papillae: 10–13 pairs ---------- 9
7. Anteriormost ventral papillae far anterior to vitellaria ---------- *C. vietnamensis*
   Anteriormost ventral papillae immediately posterior to cirrus sac -------- *C. harwoodi*

8. Body small (1.7–2.1 mm long), cirrus sac long, short esophagus, ceca without diverticula ---------- *C. rauschi*
   Body small (1.6–2.3 mm long), cirrus sac long, very short esophagus, ceca without diverticula ---------- *C. pricei*
   Body large (2.9–3.8 mm long), cirrus sac relatively short, short esophagus, ceca with diverticula ---------- *C. pakistanensis* (bird)
9. Ceca with diverticula, uterine loops 16–18, metraterm equal to or longer than cirrus sac, eggs small (16–23 μm) ---------- *C. indicus*
   Ceca with diverticula, uterine loop 18–20, metraterm equal to or longer than cirrus sac, eggs large (21–25 μm) ---------- *C. misrai*
   Ceca without diverticula, uterine loop 15–19, metraterm slightly longer than cirrus sac, eggs small (18–21 μm) ---------- *C. chinensis*
10. Ventral papillae: 6–9 pairs ---------- 11
    Ventral papillae: 8–12 pairs ---------- 12
    Ventral papillae: 12–18 pairs ---------- 13
11. Cirrus sac long, nearly 1/2 body length ---------- *C. lagunae*
    Cirrus sac short, less than 1/5 body length ---------- *C. orientalis*
12. Body small (1.6–2.4 mm), uterine loops 16–17, eggs 24–29 μm with short or long filament (15–180 μm) ---------- *C. chilinae*
    Body large (2.3–2.8 mm), uterine loops 12–17, eggs 19–24 μm with long filament (156-185 μm) ---------- *C. hatcheri*
    Body large (2.3–5.7 mm), uterine loops 12–14, eggs 25–30 μm with short filament (100-150 μm) ---------- *C. verrucosa*
13. Body small (1.1–1.5 mm), elongated, uterine loops 10, vitellaria begin posterior to mid-body ---------- *C. liara*
    Body large (2.4–7.0 mm), tongue-shaped, uterine loops 20, vitellaria begin anterior to mid-body ---------- *C. hisikui*
    Body large (3.3–6.3 mm), elongated, uterine loops 24–26, vitellaria begin posterior to mid-body ---------- *C. cygni*

In conclusion, adult flukes of the genus *Catatropis* were identified in the large intestine of Pitalah ducks in Indonesia. Morphological analyses allowed us to classify them as *C. indicus*. Further molecular research on *Catatropis* species is warranted.

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