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Systematic status of new species of *Diplotriaena ayazi n.sp.* (Nematode: Filariidae) species from Bank Myna (*Acridotheres Ginginianus*) Latham; 1872 (Passeriformes: Sturnidae) in Sindh, Pakistan's District Larkana

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Article Details

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ABSTRACT

Background: Nematodes are critical parasites that have a positive as well as negative impact. They are parasites that infiltrate humans, animals, and plants, causing ailments and financial losses. Nevertheless certain nematodes are favorable and aid in controlling pest populations, even in agricultural settings. Objectives: To expand the taxonomic comprehension of parasitic nematodes and support zoological classification, parasitology, and biodiversity research. To verify the new species' peculiarity and support its recognition as a new species (n.sp.) by evaluating it with previously reported *Diplotriaena* species. The body cavity of Bank myna *(Acridotheres ginginianus)* in District Larkana, Sindh, Pakistan, yielded a new species Diplotriaena ayazi sp. nematode. Material and methods: The conventional approach used in ornithological study and morphological analysis. To ensure the legitimacy of this study while causing the least amount of harm to individual birds, particular rules are followed to throughout the mynas' collection, preservation, and morphological analysis. Depending on the objectives of the study and the ethical dilemmas raised by animal research, several approaches are taken. The Passeriformes order includes the Mynas family of birds. They are frequently studied for zoological, taxonomic, and ecological research endeavors. Results: A total of thirty nematodes (♂♂) were noted. The following traits of the current nematode show diversity from its correspondent: body size, form, trident size and shape, spicule shape, and the absence of caudal papillae. *Diplotriaena ayazi n.sp* may be considered a new species based on these morphometrical alterations. Conclusion: However, the authoress dedicated this species in memory of Revolutionary Modern Sindhi Poet Shaikh Ayaz.

INTRODUCTION

Acridotheres ginginianus Latham [1]. A group of birds belonging to the starling family (Sturnidae) are called mynas. The Indian subcontinent's native Bank Myna *(Acridotheres ginginianus)* is essential for sustaining the environment and expanding biological study in a number of ways. This friendly and extremely adaptive bird is frequently seen close to agricultural regions and communities of humans [2]. These birds are frequently found feeding on organic waste that has been thrown in landfills, slaughter facilities, and areas close to market.

Furthermore, to control population of flies and other insect, this scavenging habit may help to reduce the quantity of recyclables and their droppings can spread seeds in plant regeneration and biodiversity [3]. Mynas dig and burrows into clay banks for nesting and helping to aerate the soil, this action may assist other species that pursue sanctuary in uncontrolled burrows [4]. The Bank Myna's spices in urban environments has led as a good indicator for habitat quality and environmental changes. Since, it harbors various nematodes especially *Diplotriaena* species may be helpful for research in the field of parasitology. These, co-relation can provide calear understanding of the transmission, variance and host of avian parasites [5]. In South Asian ecosystems, the Bank Mynas are the key species and provide an extensive range of services, including seed distribution, weed control and used as a bio-indicator and research studies. The population mynas indirectly benefits waste management, agriculture and ecological researches.

Birds may transmit variety of gastrointestinal and tissue dwelling nematode species that belongs to *Ascaridia*, *Capillaria*, *Syngamus*, and *Diplotriaena* genera. These nematodes frequently occur in the tissues, air sacs and intestine of birds. Birds are the ideal for nematodes to thrive, reproduce and to complete their life cycles [6].

A high degree of host specificity is the result of recurrent co-evolution amongst nematodes and their bird hosts. Certain nematodes are helpful in phylogenetic and evolutionary research because they are unique to particular bird groups or genera. Researchers can better comprehend the co-evolutionary interprets of parasites and their hosts because to their relationship [7]. Nematode transmission through ingestion or skin penetration can be made easier by the frequent encounters that birds have with soil, water, insects, and other possible intermediate hosts or paratenic hosts (such as earthworms and beetles). Before infecting avian hosts, *Diplotriaena* different species, for instance, finish their life cycle in intermediate insect hosts. Bird health can be greatly altered by nematode infections, which may modify fitness, behavior, and population dynamics. Bearing an eye on these infections aids in disease control, ecology, and bird conservation. The study of certain avian nematodes is of great importance for veterinary and public health because they may be zoonotic or have an impact on domesticated birds, such as poultry. Specifically in ornithological and ecological experiments, nematode infections in birds are used to evaluate avian health, ecological stress, and contamination from the environment [8]. The *Diplotriaena* nematodes is one of the filariid parasitic nematodes that mostly live in the thoracic cavity and air sacs of birds including the bank myna *(Acridotheres ginginianus)*, (Sarwar, 1956). These nematode species belong to a bigger parasite group that can affects host ecosystem and evolution and they are banquet by various vectors such as insects [25]. The *Diplotriaena* species can cause severe respiratory infections like anemia, skinniness and dyspnea. They cause inflammation and obstruction of air sacs and severe or chronic infections can be lethal especially in children with compromised immune system [26].

The deficient immune response and respiratory function are widespread secondary infections. These infections may cause lethargy, reduced vocal activities, changes in feeding behaviors and impaired flight performance in birds. These behavioral changes may reduce mating success thus affecting individual fitness and survival [27]. *Diplotriaena* species may cause diverse physiological performances than gastrointestinal parasites such as *Ascaridia* or *Capillaria* because they dwell into the respiratory system. *Diplotriaena* species occupy deeper air sacs, producing less evident but more enduring effects than *Syngamus* trachea, which lodges in the trachea [28]. These parasites are ecological indicators of host susceptibility, vector abundance, and environmental health. Infections with *Diplotriaena* can affect the dynamics of bird populations by decreasing host longevity and reproductive success. By raising juvenile mortality or decreasing adult reproductive output, high parasite burdens might cause population decline or change demographic patterns [29]. Stress and environmental changes can intensify parasite consequences in fragmented habitats, raising conservation implications for both urban and rural bird populations [30]. Bank myna infections with *Diplotriaena* can be tracked as a bioindicator of ecological disturbance. Assessing the health of avian population requires an understanding of parasite stresses, especially in areas where human activity has changed the landscape. To lessen long-term effects on bird populations, conservation techniques should combine habitat management and parasite surveillance.

MATERIALS AND METHODS

COLLECTION OF SPECIMENS

Rather than using random sample, the sampling strategy used in this study was based on convenience sampling. During field visits, birds (*Acridotheres ginginianus*) were opportunistically captured from the study area based on accessibility and availability.

The design of this study is observational and descriptive. Without any kind of manipulation or interference, it entails the analysis and recording of parasitic nematodes (*Diplotriaena* spp.) that have been obtained from naturally infected *Acridotheres ginginianus*. The objective was to characterize the parasites' physical traits, prevalence, and host relationships as shown in specimens that were gathered from the field.

During necropsy or fecal investigation, nematode specimens were taken from the host organisms (such as birds, amphibians, reptiles, and mammals). Birds were only be euthanized or subjected to necropsy by the justified scientific method justified and no viable non-lethal alternatives exist. Every procedure involving birds was carried out in compliance with national and institutional standards for the handling and care of research animals.

Currently, Eleven (11%) of the 100 *Acridotheres ginginianus* specimens that were analyzed were determined to be infected. Thirty (30) species of *Diplotriaena* nematode individuals were collected from these infected hosts, suggesting a significant parasite load and prevalence in the studied population.

FECAL INVESTIGATION

On a microscope slide, a tiny quantity of excrement is combined with saline and viewed under a microscope. Although this approach is rapid, it might not be sensitive enough to detect low-level infections.

NECROPSY

There are two methods of necropsy internal as well as external necropsy.

EXTERNAL NECROPSY

Analyzing any apparent lesions, feather quality, and body condition.

INTERNAL NECROPSY

According to institutional rules for animal care, the hosts were either precisely euthanized or newly died. Nematodes have been monitored for in the gastrointestinal system and other significant tissues.

Respiratory System: Opening the thoracic cavity to check for inflammation, adult worms, or other abnormalities in the lungs and air sacs. The presence of *Diplotriaena* species in the air sacs frequently reults in thickening and turbidity.

Digestive System: Checking for indications of parasites or other secondary infections in the gastrointestinal tract.

Other Organs: Checking for growth, discoloration, or other anomalies in the kidneys, liver, or spleen that might point to systemic parasite effects.

Gathering tissue samples for histopathological analysis and keeping any parasites obtained in suitable fixatives for identification is known as sample collection.

PRESERVATION AND FIXATION

After cleaning host debris with physiological saline (0.9% NaCl solution), collected nematodes had been preserved in either hot 70% ethanol or 4% formalin. Some specimens were prepared for temporary mounts in glycerin or lactophenol for morphological analysis.

MORPHOLOGICAL EXAMINATION

A compound microscope was used for looking at the specimens. An ocular micrometer that had been calibrated was used to gather measurements. The digital imaging systems or camera lucida were used to create the illustrations and micrographs. Important physical characteristics were described in detail, notably the tail, esophagus, reproductive organs, and buccal capsule.

IDENTIFICATION

Standard taxonomic keys and descriptions from earlier investigations by (Yamaguti, S. [9], Anderson, R. C. [10], Chabaud, A.G. [11], Gibbons, L.M. [12] and Baylis, H.A. & Daubney, R. [13] were used for determining nematodes. To verify authenticity or prove distinctiveness. A comparative investigation with closely related species was undertaken.

DEPOSITION OF SPECIMENS

For future use, type specimens were submitted in reputable zoological or parasitological museums of University of Sindh, Jamshoro, Pakistan

RESULTS

Family: Diplotriaenidae (Skrjabin) [14]

Genus: *Diplotriaena* (Railliet and Henry) [15]

Species: *Diplotriaena ayazi n.sp*

Host: *Acridotheres ginginianus* Latham

No. of specimens recovered: 30

No. of hosts found positive: 11

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Parasitic habitat: Body cavity

Locality: District Larkana, Sindh, Pakistan

The worm's elongated, robust, and very muscular body reaches (12.43-12.47 x 0.31-0.26) mm, rounded from front to back. There are two tridents, one with a rounded tip and the other with a pointy tip, and they are both unique in size and shape. The prongs of the left and right tridents are not equal and measure (0.14 x 0.07) mm. The left spicule, which emerges outside the posterior extremity with a little curvature measuring (0.32) mm, is smaller than the right spicule. The right spicule, which grows outside the posterior apex and has a broad curve curving downward, is larger than the left spicule, measuring (1.64) mm.

FIGURE: 2. BANK MYNA *(ACRIDOTHERES GINGINIANUS)* LATHAM, 1790 [1] FROM WHICH *DIPLOTRIAENA AYAZI* WAS RECORDED.

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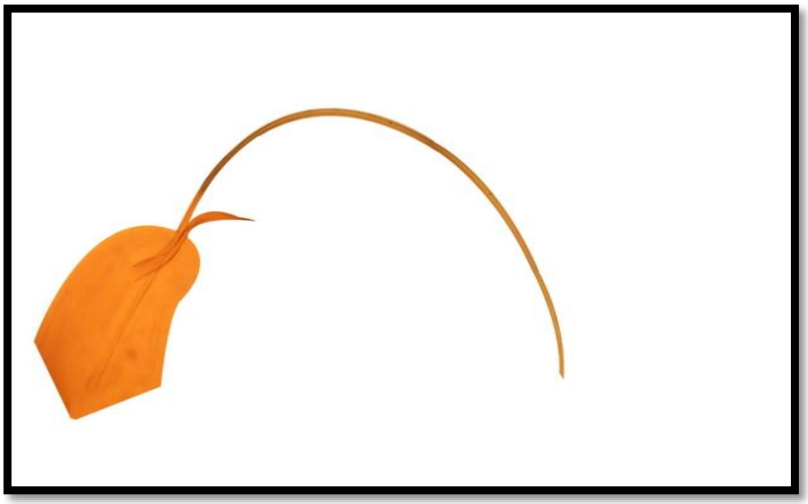
(i)



(ii)

FIGURE 3 *DIPLOTRIAENA AYAZI N.SP.* (♂♂). (I) CAPTURED VIEW OF ANTERIOR PORTION (II) CAPTURED VIEW OF POSTERIOR PORTION.

TABLE. (1) COMPARING THE TRAITS OF SEVERAL SPECIES OF THE GENUS *DIPLOTRIAENA* THAT HAD BEEN GATHERED FROM VARIOUS AVIAN HOSTS BY RAILLIET AND HENRY IN 1909. ND=NOT DETECTABLE MILLIMETERS ARE USED TO MEASURE THE PARAMETERS

S.NO	Parameters	Present species	*D. yusufi*	*D. niltavae*	*D. bargusinica*	*D. tristisi*
1.	Body length	12.43-12.40 *mm*	40.2 *mm*	24.0-24.4 *mm*	31.0-46.4 *mm*	32.94 *mm*
2.	Body width	0.31-0.26 *mm*	0.39 *mm*	0.496-0.512 *mm*	0.72-0.77 *mm*	0.455 *mm*
3.	Tridents	0.14-0.11 x 0.15-0.07 *mm*	0.10-0.07 x 0.11-0.07*mm*	0.128-0.144 *mm*	0.13-0.14*mm*	0.13 *mm*
4.	Caudal papillae	ND	1-6	1-12	1	1-9
5.	Left spicule	1.64 *mm*	1.59 *mm*	0.656-0.720 *mm*	0.64-0.66 *mm*	2.39 *mm*
6.	Right spicule	0.32 *mm*	0.37 *mm*	0.46-0.49 *mm*	0.38-0.5 *mm*	0.58 *mm*
7.	Host	*Acridotheres tristis*	*Acridotheres tristis*	*Niltava grandis*	*Turdus roficollis*	*Acridotheres tristis*
8.	Enviroment	Aantomical cavity	Aantomical cavity	Aantomical cavity	Aantomical cavity	Aantomicacavity
9.	Locality	District Larkana Sindh, Pakistan	District Larkana, Sindh, Pakistan	Uttarakhand	Bhutan	Burdwan

DISCUSSION

The current work offers fresh perspectives on the taxonomy of nematode and field of Parasitology. *Diplotriaena ayazi* differs significantly from earlier research. This result advances our knowledge of biological, ecological, taxonomic and parasitological status. Based on

Majumdar and Chakravorty's report on the *Sturnus contra* of Burden, *D. sternopastori* reflected difference from *Diplotriaena ayazi* in that its body length and its maximum breadth; its tridents which are shorter in length; and its left spicule which is longer than its right [16]. In *Acridotheres tristis* of Burden, Majumdar and Chakravorty noticed that *D. tristisi* distincted from *Diplotriaena ayazi* in body length and its maximum breadth; shorter tridents; and its left spicule is longer than its right [16]. Majumdar and Chakravorty described *D. molpastisi* in *Acridotheres ginginianus* of Burden. In contrast to *Diplotriaena ayazi*, this species has a maximum body length, longer tridents, and a longer left spicule than a right [16]. According to Dysarkar S. R. and Debabrata Sen (*D. chapwatensis)* species of *Myiophoneus caeruleus temmincki* found in India and it is dissimilar from *Diplotriaena ayazi* possess larger body with maximal breadth, equal prongs and a lengthier length, it's left side spicule is longer than its right side [17]. While in contrast to *Diplotriaena ayazi*, *D. zootherae*, as described in Zoothra citrine in Uttarakhand vary by its longer left side spicule as compared with its right spicule, equal sized pointed prongs having and widest body [17]. Debabrata Sen and Dysarkar S. R. has conducted a research on *Niltava grandis grandis* in Uttarakhand, India as well as finding of *D. Nitavate* described that, in contrast to *Diplotriaena ayazi*, this species is notable by its larger body length and maximum width, equal prongs and shorter body length and longer left side spicules as compared to right side spicules [17].

In Nagpur, Baylis H. A, found *Acridotheres tristis* to harbor *D. nagpurensis* and differ from *Diplotriaena ayazi* having a longer side spicule than right spicule, longer tridents and bigger body length with maximum body width [19]. According to Baylis H. A. *Acridotheres tristis* from Nagpur *D. Tricuspus*, was differ from *Diplotriaena ayazi* in lengthier and wider body parts, shorter tridents and a longer left spicule than right spicules [19]. In Burma, *D. bhamoensis* Baylis H. A. has found in *Ethiopsar albocinclus*. It has maximal body width and length were longer than *Diplotriaena ayazi* [19]. The *D. graculi* Baylis H. A., observed in *Pyrrhocorax*, Calcutta, differ from *Diplotriaena ayazi* in its larger body length and maximum body width, left spicule observed longer than right spicule [19]. The *D. dubia* Baylis H. A. discovered in *Pyrrhocorax* in Calcutta, which was different from the existing *Diplotriaena ayazi*. Its width is maximum and its body is larger, the left spicule found longer than the right spicule [19]. Baylis H. A., in 1939, *D. urocissae* has been identified in *Calcuta's Urocissa flavirostris*. It was differed from *Diplotriaena ayazi*, the left spicule is longer than the right and its body length found bigger and its width is at its maximum [19]. Passer species found in Pakistan's *Passer domesticus* and *Passer pyrrhonotus* by Dharejo A. M., Chandio I. and Naz S.D. with shorter trident, left spicule longer than its right spicule and its body observed shorter in comparison to *Diplotriaena ayazi* [15]. Central and Northern Colorado is home of species of *D. lagopusi.* According to Olsen O. W. and Braun C. E., it differs from *Diplotriaena ayazi* has longer tridents, longer left and right spicules and bigger body length and maximum width [20]. *Diplotriaena ayazi* may compared from the Canadian species *D. andersoni*, identified by Olsen O. W. and Braun C. E., possess a longer left spicule and bigger body length and maximum body width [20]. In United States, Henri and Seibert discovered *D. thomasi* in *Zonotrichia albicollis lllinois.* This difference varies from *Diplotriaena ayazi* has longer body, shorter trident and longer left spicule than right spicule [21]. Bilqees F. M. and Jehan N. state that *D. streptopelia* is referred to as *Streptopelia senegalensis* in Pakistan.

This species differed from *Diplotriaena ayazi* in that its body is longer; its right spicule is longer than left spicule [22]. According to Wong P. L., Anderson R. C., and Frimeth J. *D. utae* which was discovered in *Perisoreus canadensis*, this type differed from *Diplotriaena ayazi* in that it has a larger body length and maximum body width, a longer trident, and longer left and right spicules [23]. According to *D. yusufi* Soomro B, Ghachal G. S, and Shaikh M. Y. *et al.*, a variant of *Acridotheres tristis* was found in Sindh, Pakistan. It is different from *Diplotriaena ayazi* in that it has a larger body length and breadth, longer tridents, six caudal papillae, and a longer right spicule than a shorter left [24].

Even while the current species' measurements of body length and trident size somewhat like those of *D. yusufi*, *D. niltavae*, *D. bargusinica, and D. tristisi*, this kind of variation is frequent among closely related taxa. When certain morphological, ecological, and geographic characteristics are taken into account, morphometric overlap by itself does not invalidate species differentiation. In comparison to all other mentioned species, particularly *D. yusufi* (40.2 mm) and *D. bargusinica* (31.0–46.4 mm), the current species is distinguished by a noticeably shorter body length (12.40–12.43 mm). Compared to other species, trident size is likewise in the lower range, but it can vary in shape and structure, which are characteristics that are frequently more taxonomically important than size alone.

The length of the left spicule (1.64 mm) is the most conclusive diagnostic feature among all assessed parameters. It is significantly longer than that of *D. niltavae* (0.656–0.720 mm) and *D.*

bargusinica (0.64–0.66 mm), and it is second only to *D. tristisi* (2.39 mm). Because of its reproductive purpose and structural complexity, spicule shape is regarded as a very trustworthy characteristic in worm classification. The current species' unique connection with *Acridotheres tristis* in a geographically limited locality (District Larkana, Sindh, Pakistan) further supports its differentiation from other congeners by indicating ecological and biogeographical isolation.

STATISTICAL ANALYSIS

A structured data analysis method was put into place in order to improve the morphological comparisons between *Diplotriaena* species. First, the Shapiro-Wilk test was used to determine whether continuous morphometric variables (such as body length, body width, trident dimensions, and spicule sizes) were normal. One-way ANOVA was utilized to assess statistically significant species differences for data that was normally distributed. Non-parametric Kruskal–Wallis tests were used when the homogeneity of variance or normality criteria were not satisfied.

Independent sample t-tests with Bonferroni correction to account for Type I error were used to further evaluate pairwise comparisons across species for specific features (such as spicule size and trident dimensions). For every comparison, p-values were computed and published in order to establish statistical significance ($p < 0.05$).

Using morphometric variables, a Principal Component Analysis (PCA) was carried out to confirm overall morphological differentiation among species. The most important characteristics for taxonomic categorization were identified and inter-species separation was visualized thanks to this multivariate technique

Regarding host prevalence and geographic differences, prevalence was calculated as the proportion of infected hosts out of the total studied. To evaluate variations in infection rates among sites, a chi-square test was utilized.

Additionally, one-way ANOVA and post-hoc Tukey testing were used to compare caudal papillae and spicule size in order to evaluate interspecies variance. The length of the left spicule and the quantity of caudal papillae are two of the most important differentiating characteristics, according to these investigations.

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TABLE # 2: COMPARISON OF MORPHOMETRIC TRAITS AMONG *DIPLOTRIAENA* SPECIES

Parameter	F-Statistic (F)	Degrees of Freedom (df)	p-value	Interpretation
Body length	22.18	4, 45	<0.001	Significant difference between species
Body width	18.67	4, 45	<0.001	Significant
Trident size	6.91	4, 45	0.0002	Significant
Left spicule	28.34	4, 45	<0.001	Highly significant
Right spicule	14.72	4, 45	<0.001	Significant
Caudal papillae*	—	—	—	Not analyzed

*Note: Caudal papillae analysis excluded due to incomplete or qualitative data. (Because of absence)

INTERPRETATION

The use of spicule lengths and body size as conclusive morphological markers for species identification is justified by the statistically significant variance observed in all quantitative parameters (with the exception of caudal papillae) among species, indicating their relevance for taxonomic differentiation.

CONCLUSION

In conclusion, even though some morphometric characteristics, such body length and trident size, overlap, their taxonomic value is strengthened when compared to host specificity, regional distribution, and spicule dimensions. In the current analysis, these characteristics—in particular, spicule morphology and host association—offer the strongest foundation for species-level identification. This *Diplotriaena ayazi* is regarded as a new species that is interesting to the field of taxonomy and parasitology to its morphometric plurality.

The discovery of *Diplotriaena* nematode species in *Acridotheres ginginianus* from District Larkana, Sindh has significantly amplified parasitological records of host birds from South Asia. This will expand host and topographical range of the genus *Diplotriaena*. This finding highlights the standing of region-specific in the field of parasitological research, that uncovering hidden biodiversity. The intricacy and taxonomic diversity of the genus has demonstrated with morphological caricaturists, mainly in the length of spicule and tridents, host association and potential diversity in structure.

The scientific perspective of this discovery will un fold new avenues researchers to study host-parasite coevolution, transmission dynamics and effects of ecological and environmental factors for parasitic prevalence in birds. Furthermore, it will increase the possibility in findings of cryptic species within *Diplotriaena*,

In relations to broader implications of understanding nematodes' life cycles and pathogenicity, it is crucial for upholding biodiversity and to protect health of various species of birds. Parasitic infections can have significant effects on bird's populations, mainly in stressed or declining species. For conservation projects involving migratory or vulnerable bird species a baseline data for avian health is obtained by documenting these parasites.

Since some bird species such as *Acridotheres ginginianus* in agroecosystem serves as biological indicators or pest vectors and data of their parasites may aid in the development of integrated pest management (IPM) strategies. Nematode infections may indirectly impact crop protection strategies, avian pest control, and ecological interactions if they have an impact on host behavior, reproduction, or population dynamics.

In conclusion, in addition to being taxonomically noteworthy, this discovery offers fundamental information that may impact upcoming studies in conservation biology, pest management, and bird parasitology. Our knowledge of parasitic nematodes and their function in bird ecology must be expanded via ongoing surveillance and comparative research.

NOVELTY OF SPECIES

To the best of my knowledge, the scientific literature does not currently contain any species with the name *Diplotriaena ayazi*. In District Larkana, Sindh, Pakistan, a species called *Diplotriaena ayazi* has been identified as a new species that differs from the rest species. The distinctive physical traits that set this species apart from other species in the genus *Diplotriaena* led to its identification.

The factor supports the uniqueness for *Diplotriaena ayazi*., the distinct morphological features Unlike other known *Diplotriaena* species, this one has distinct morphological features, such as particular trident, spicule, and egg measurements and structures.

In conclusion, even though some morphometric characteristics, such body length and trident size, overlap, their taxonomic value is strengthened when compared to host specificity, regional distribution, and spicule dimensions. In the current analysis, these characteristics—in particular, spicule morphology and host association—offer the strongest foundation for species-level identification. This *Diplotriaena ayazi* is regarded as a new species that is interesting to the field of taxonomy and parasitology to its morphometric plurality.

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