



## “STUDIES ON MORPHOTAXONOMY OF *AZYGIA INDICA SP.NOV.* FROM *CHANNA MARULIUS*”

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### ABSTRACT

Present investigation deals with a new species of the genus *Azygia* Looss, 1899 from the intestine of *Channa marulius* from Bhokar, Loha, Kandhar, Nanded (M.S.) India were found infected with One Hundred Twenty (120) trematode parasites during June, 2019 to May, 2021. The new species *Azygia indica Sp.Nov.* comes closer to all known species of the genus *Azygia* in general topography of organ but differs due to body is elongated, medium, non-spinose, dorsoventrally flattened with rounded extremities, oral sucker sub-terminal, spherical, ventral sucker larger than oral suckers, spherical, pre-equatorial, Pre-pharynx absent, Pharynx muscular, immediately behind oral suckers, Oesophagus oval, muscular, Intestinal caeca broad, slightly sinuous, runs alongside of the body, genital pore pre-acetabular, excretory pore lies at posterior end of body, testes are oval in shape and situated side by side, preovarian, cirrus pouch elongated, lying at anterior side of ventral suckers, cirrus muscular, tubular, within cirrus pouch, forms vas deferens, Vas deferens short tube, bifurcates and forms vasa efferentia, vasa efferentia are long, runs posteriorly and reaches to testes, ovary compact, lies lateral to posterior to testis, vitellaria are follicular, small, extending from ventral sucker, uterus arises from ootype, lying transversely between ovary and posterior border of ventral sucker, eggs elongated, oval, non-operculated.

**Key words-** *Azygia indica Sp.Nov.*, *Channa marulius*, Trematoda.

### INTRODUCTION

*Azygia* Looss, 1899, a genus of digenetic trematode (Azygiidae), has been reported from Europe, North America, Japan, India, China and the Philippines. The genus seems to present a great deal of confusion (Manter, 1926; Stunkard, 1956; Wootton, 1957; Gibson, 2002), with differing judgments regarding what constitutes species-level differences. Manter (1926) synonymized *Azygia loossii* Marshall and Gilbert, 1905 with *Azygia angusticauda* and 7 other names with *Azygia longa* (Leidy, 1851), and along with *Azygia acuminata* Goldberger, 1911 considered the genus to have 3 representative species in North America. Van Cleave and Mueller (1934) suggested that *Azygia acuminata* was a synonym of *Azygia longa*. However, Stunkard (1956) rejected this decision, re-erected *Azygia sebago* Ward, 1910 from Manter's synonymy with *Azygia longa*, and suggested that *Azygia acuminata* was a synonym of *Azygia sebago*. Wootton (1957) resurrected *Azygia acuminata*, studied the life cycle of this worm, and

provided a dichotomous key to the species he considered valid. Much of the confusion in this genus surrounds the assumption that members of these species can infect multiple host species and that they display sometimes massive differences in some morphological traits because of this.

Despite this overall confusion, and the synonymy with *Azygia loossii* described above, the identity of *Azygia angusticauda* as a distinct form has been recognized by all previous authors (Wootton, 1957). In all other species of *Azygia* in North America, the gonads are as close or closer to midbody as is the ventral sucker, i.e., both the gonads and the ventral sucker are situated very far to the anterior. In contrast, specimens of *Azygia angusticauda* possess gonads and a ventral sucker positioned posteriorly, i.e., the gonads are close to the posterior end and the ventral sucker is close to midbody. This difference is apparently not the result of differential growth in different regions of the body (at least, not entirely) because both Sillman (1953) for *Azygia longa* and Stunkard (1956) for *Azygia sebago* diagrammed juvenile worms with the ventral sucker and gonads to the anterior, as in the adult forms. Dawes (1947) however, recognizes only a single species (*Azygia lucii*) in Europe. *Azygia hwangtsiyui* Tsin, 1933 (Syn. *Azygia amurensis* Zmeev, 1936) was reported and redescribed by F. Moravec and O. Sey, 1989 (Vietnamese specimens). Then *Azygia angusticauda* (Stafford, 1904) {Bhalerao, 1942} was redescribed by of Bhure et.al., 2010. Later on Michael A. Barger, 2014 reported *Azygia aphredoderid*. Then *Azygia gigantea* was added by Gaikwad et.al., 2019 from *Channa punctata*. Recently Khillare et.al., 2022 reported *Azygia acetabulata* from *Channa punctatus* at Wankhed, Tehsil Sangrampur, Dist. Buldhana (M.S.) India.

### MATERIAL AND METHODS

During the survey of Piscean Helminths, Eighty Six (86) out of Two Hundred Forty (240) freshwater fish *Channa marulius* from Bhokar, Loha, Kandhar, Nanded (M.S.) India were found infected with One Hundred Twenty (120) trematode parasites during June, 2019 to May, 2021. These trematodes were preserved in 4% hot formalin, Five specimens are stained with Harris haematoxylin and Borax carmine, dehydrated through various ascending alcoholic grades (30 %, 50%, 70 %, 90 %, 100 %), cleared in xylene, mounted in D.P.X. and drawings are made with the aid of Camera Lucida. All measurements are recorded in millimeters unless otherwise stated.

### RESULTS (Description based on Five specimens)

The body is elongated, medium, non-spinose, dorsoventrally flattened with rounded extremities and measures 22.12 millimeters (21.75 millimeters to 22.50 millimeters) in length and 5.20 millimeters (3.80 millimeters to 6.60 millimeters) in width. The oral sucker is sub-terminal, spherical and measures 0.951 millimeters (0.914 millimeters to 0.988 millimeters) in length and 1.01 millimeters (0.892 millimeters to 1.128) in width. The ventral sucker is larger than oral suckers, spherical, pre-equatorial and measures 2.451 millimeters (1.996 millimeters to 2.906 millimeters) in length and 2.496 millimeters (1.998 millimeters to 2.995 millimeters) in width. Pre-pharynx absent. Pharynx muscular, immediately behind oral suckers and measures 0.088 millimeters (0.082 millimeters to 0.095 millimeters) in length and 0.114 millimeters (0.096 millimeters to 0.132 millimeters) in width. Oesophagus is oval, muscular and measures 0.642 millimeters (0.607 millimeters to 0.677 millimeters) in length and 0.398 millimeters (0.371 millimeters to 0.426 millimeters) in width. Intestinal caeca broad, slightly sinuous, runs along side of the

body. The genital pore pre-acetabular, measures 0.146 millimeters (0.125 millimeters to 0.168 millimeters) in length and 0.055 millimeters (0.035 millimeters to 0.075 millimeters) in width. The excretory pore lies at posterior end of body and measures 0.196 millimeters in diameter. The testes are oval in shape and situated side by side, preovarian. The right (slightly posterior) testis is smaller than left testis (Anterior testis), measures 0.860 millimeters (0.812 millimeters to 0.909 millimeters) in length and 0.680 millimeters (0.614 millimeters to 0.747 millimeters) in width and lies at 16.25 millimeters from anterior extremity. The Left (Anterior) testis measures 1.002 millimeters (0.893 millimeters to 1.111 millimeters) in length and 0.577 millimeters (0.469 millimeters to 0.685 millimeters) in width and lies at 15.75 millimeters from anterior extremity. The cirrus pouch is elongated, lying at anterior side of ventral suckers and measures 1.227 millimeters (1.132 millimeters to 1.323 millimeters) in length and 0.385 millimeters (0.318 millimeters to 0.453 millimeters) in width and lying at 3.25 millimeters from anterior extremity. The cirrus is muscular, tubular, within cirrus pouch, forms vas deferens and measures 1.160 millimeters (1.106 millimeters to 1.214 millimeters) in length and 0.034 millimeters (0.025 millimeters to 0.044 millimeters) in width. Vas deferens short tube, bifurcates and forms vasa efferentia and measures 2.911 millimeters (2.882 millimeters to 2.941 millimeters) in length and 0.044 millimeters (0.029 millimeters to 0.059 millimeters) in width. The vasa efferentia are long, runs posteriorly and reaches to testes and measures 5.884 millimeters (5.845 millimeters to 5.924 millimeters) in length.

The ovary is compact, lies lateral to posterior to testis, it measures 1.019 millimeters (0.693 millimeters to 1.345 millimeters) in length and 1.558 millimeters (1.123 millimeters to 1.994 millimeters) in width and lies at 17.50 millimeters from anterior extremity. Receptaculum seminis absent. The oviduct arises from the ovary and opens at the ootype. The vitellaria are follicular, small, extending from ventral sucker. The uterus arises from ootype, lying transversely between ovary and posterior border of ventral sucker and measures 7.589 millimeters (7.423 millimeters to 7.756 millimeters) in length and 2.923 millimeters (2.483 millimeters to 3.363 millimeters) in width. The eggs elongated, oval, non-operculated and measures 0.091 millimeters (0.088 millimeters to 0.095 millimeters) in length and 0.035 millimeters (0.027 millimeters to 0.044 millimeters) in width.

### DISCUSSION

*Azygia* Looss, 1899, a genus of digenetic trematode (Azygiidae), has been reported from Europe, North America, Japan, India, China and the Philippines. The genus seems to present a great deal of confusion (Manter, 1926; Stunkard, 1956; Wootton, 1957; Gibson, 2002), with differing judgments regarding what constitutes species-level differences. Manter (1926) synonymized *Azygia loossii* Marshall and Gilbert, 1905 with *Azygia angusticauda* and 7 other names with *Azygia longa* (Leidy, 1851), and along with *Azygia acuminata* Goldberger, 1911 considered the genus to have 3 representative species in North America. Van Cleave and Mueller (1934) suggested that *Azygia acuminata* was a synonym of *Azygia longa*. However, Stunkard (1956) rejected this decision, re-erected *Azygia sebago* Ward, 1910 from Manter's synonymy with *Azygia longa*, and suggested that *Azygia acuminata* was a synonym of *Azygia sebago*. Wootton (1957) resurrected *Azygia acuminata*, studied the life cycle of this worm, and provided a dichotomous key to the species he considered valid. Much of the confusion in this genus surrounds the assumption that members of these species can infect multiple host species and that they display sometimes massive differences in some morphological traits because of this.

Despite this overall confusion, and the synonymy with *Azygia loossii* described above, the identity of *Azygia angusticauda* as a distinct form has been recognized by all previous authors (Wootton, 1957). In all other species of *Azygia* in North America, the gonads are as close or closer to midbody as is the ventral sucker, i.e., both the gonads and the ventral sucker are situated very far to the anterior. In contrast, specimens of *Azygia angusticauda* possess gonads and a ventral sucker positioned posteriorly, i.e., the gonads are close to the posterior end and the ventral sucker is close to midbody. This difference is apparently not the result of differential growth in different regions of the body (at least, not entirely) because both Sillman (1953) for *A. longa* and Stunkard (1956) for *A. sebago* diagrammed juvenile worms with the ventral sucker and gonads to the anterior, as in the adult forms.

The present new form *Azygia indica* Sp.Nov. comes closer to known valid species of the genus *Azygia* Looss, 1899 in general topography of organs but differs from *Azygia pristipomai* Tubangui, 1928 in having body 1.93-3.2×0.86-1.00mm, oral suckers 0.34-0.45 mm, acetabulum 0.31-0.38mm, pharynx 0.16-0.18mm, eggs 0.066-0.068×0.040-0.044mm.

The *Azygia indica* Sp.Nov. differs from *A. hwangtsiyui* Tsin, 1933 (Syn. *Azygia amurensis* Zmееv, 1936) as reported by F. Moravec and O. Sey, 1989 (Vietnamese specimens) in having body 3.94-5.93×0.68-1.01mm, oral sucker 0.435-0.798×0.530-0.770mm, acetabulum 0.435-0.558×0.435-0.612mm, pharynx muscular, 0.177-0.245×0.177-0.258mm, oesophagus 0.068mm, caeca extending posteriorly to short distance in front of body end, ovary and testes oval or of irregular shape, close to each other, situated in posterior half of body, ovary 0.190-0.299×0.231-0.299mm, anterior testis 0.204-0.245×0.340-0.408mm, posterior testis 0.285-0.340×0.313-0.326mm, cirrus sac spherical, 0.204-0.258×0.136-0.204mm, situated medially, uterine loops filling up intercaecal space between genital pore and ovary, vitellaria multifollicular, reaching posteriorly usually to mid distance between posterior testis and body end, eggs 0.060-0.066×0.030-0.042mm and recorded from intestine of *Ophicephalus maculates* (Channidae).

The new form *Azygia indica* Sp.Nov. differs from *Azygia sebago* (Ward, 1910) reported by Horace W. Stunkard, in having body 10×0.7-1.0mm, oral suckers 0.68mm, acetabulum distinctly smaller.

*Azygia indica* Sp.Nov. differs from *Azygia longa* (Leidy, 1851) based on description of Linton, 1940 in having body 2.10-7.9×0.42-0.91mm, oral suckers 0.31-0.63 mm, acetabulum 0.25-0.46mm, pharynx 0.11-0.25mm, eggs 0.042×0.025mm.

The present form *Azygia indica* Sp.Nov. differs from *Azygia angusticauda* (Stafford, 1904) {Bhalerao, 1942} based on description of Bhure et al., 2010 in having muscular pharynx, elongated cirrus pouch, absence of oesophagus, Ovary posttesticular, gonads lies posterior third of body, vitellaria from middle of ventral sucker to hind end of body, eggs are elongated.

The *Azygia indica* Sp.Nov. differs from *Azygia aphredoderi* Michael A. Barger, 2014 in having Body short, robust, tapering towards posterior end, 1,380 (1,145–1,855) long, 356 (298–435) wide at widest point, near ventral sucker, Oral sucker subterminal, 233 (191–284) long, 250 (213–298) wide; prepharynx absent; pharynx 88 (75–106) long, 93 (78–110) wide; oesophagus very short; ceca convoluted anteriorly, medial and dorsal to vitellaria in

posterior portion of body, terminating 118 (76–176) from posterior end, Ventral sucker at midbody, 547 (450–710) from anterior end, 259 (220–303) long, 275 (223–332) wide, Excretory bladder terminal 132 (100–186) long, Gonads contiguous, located in posterior quarter of body, varying in shape from circular to subtriangular. Testes tandem, median in posterior half of hind body; anterior testis 67 (53–76) long, 95 (69–123) wide; posterior testis 83 (68–110) long, 91 (62–120) wide. Seminal vesicle and pars prostatica tubular, contained within prostatic sac immediately anterior to ventral sucker or slightly overlapping, 87 (67–108) long, 82 (69–94) wide; common gonopore empties into sinus with broad, flat opening, Ovary immediately pretesticular, 78 (65–86) long, 93 (76–107) wide. Vitellaria follicular, lateral and extra-cecal, extending from posterior 1/3 of ventral sucker to 118 (76–176) from posterior end; vitelline reservoir dorsal to ovary; Mehlis' gland in region of ovary, usually slightly anterior, but obscured by eggs and difficult to observe, Laurer's canal and seminal receptacle not observed, but presumed present (Gibson, 2002), Uterus loops anterior of ovary, filling space between caeca; eggs 53 (46–60) long, 29 (24–34) wide and reported from *Aphredoderus sayanus* (Gilliams, 1824) Menard Creek, Menard Creek Corridor Unit, Big Thicket National Preserve, Polk County.

The *Azygia indica* Sp.Nov. morphologically differs from *Azygia gigantea* was added by Gaikwad et.al.,2019 due to body elongated, medium, non-spinose, dorsoventrally flattened with rounded extremities, measures about 14.278 (13.167-15.390) mm × 3.947 (3.078-4.816) mm. Oral sucker small, sub-terminal, spherical, muscular, and measuring 0.940 (0.855-1.026)mm × 0.883 (0.798-0.969)mm in size and lies at 0.256 mm from anterior extremity. Ventral sucker larger, oval, pre-equatorial, 1.695 (1.425-1.966) mm × 1.359 (0.997-1.710) mm in size and lies at 2.337 mm from anterior extremity. Pre-pharynx absent. Pharynx muscular, measuring about 0.071 mm × 0.042 mm. Oesophagus small, about 0.199 mm × 0.313 mm, The genital pore pre-acetabular, small, measuring about 0.071 mm × 0.042 mm in size and lies at 2.556 mm from anterior extremity. Testes oval, lies side by side, pre-ovarian. Anterior testis slightly larger and sized about 0.826 mm × 0.798 mm, lies at 6.099 mm from anterior extremity. Posterior testis slightly smaller, about 0.983 mm × 0.584 mm, locate at 6.156 mm from anterior extremity. Cirrus pouch elongated, pyriform, pre-acetabular, about 0.598 mm × 0.185 mm, lying at 2.109 mm from anterior extremity. Vas deferens short tube, bifurcates to forms vasa efferentia, measures 0.427 mm × 0.057 mm. Ovary compact, post testicular, sized about 0.954 mm × 1.496 mm, situated or lies at 8.179 mm from anterior extremity. Vitellaria follicular, Uterus arises from ootype, lying transversely between ovary and posterior border of acetabulum, measuring about 3.519 × 1.995 mm.

The present form differs from *Azygia acetabulata* Khillare et.al.,2022 due to Body short, tube shaped, non spinous, dorsoventrally leveled, measure 7.500 mm long and 2.850 mm in width. Oral sucker is little and present encompass the tip piece of the body. It is oval in shape and measures 0.259 mm long and 0.412 mm in width. Pharynx is medium behind the oral sucker, Oesophagous is exceptionally short, Digestive caeca medium, cylinder like and goes through the body and end close to rear finish of body, ventral sucker or hip bone socket is bigger than oral sucker, which oval in shape, Excretory pore at the back finish of the body, bladder 'y' molded, stretch out dependent upon somewhat back to rear testis, Testicles enormous, rounded, spread center to back finish of the body,

front testis somewhat more modest than back testis, Cirrus pocket lengthened, present before ventral sucker, Genital pore present before ventral sucker, Ovary oval in shape, pre-testicular, somewhat covering uterus, Receptaculum seminis present before ovary, Vitellaria are follicular and organized on different sides from ventral sucker to rear finish of body. Uterine loop, present transitionally among ovary and back line of ventral sucker, Eggs are oval, operculated.

These distinguishing characters are valid enough to erect a new species for this fluke and hence the new species is designated as *Azygia indica* Sp.Nov. The present species is named on account of country name.

#### TAXONOMIC SUMMARY

- Genus** : *Azygia* Looss, 1899  
**Species** : *Azygia indica* Sp.Nov.  
**Type Host** : *Channa marulius*  
**Habitat (Site)** : Stomach and Intestine  
**Type Locality** : Bhokar, Loha, Kandhar, Nanded (M.S.) India.  
**Prevalence** : One Hundred Twenty (120) mature trematodes collected from Eighty Six (86) infected host out of Two Hundred Forty (240) examined.  
**Period of collection**: June, 2019 to May, 2021.  
**No. of Specimen** : 120  
**Accession number** : PGDZ/YMN/1-05/ June, 2019 to May, 2021  
**Deposition** : Research and PG Department of Zoology, Yeshwant Mahavidyalaya, Nanded.  
**Etymology** : The species is named on account of Country name.

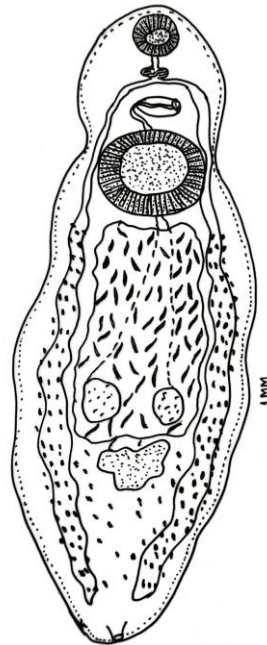


Fig.-1. Camera Lucida sketch of *Azygia indica* Sp.Nov.

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**REFERENCES**

- BARGER, MICHAEL A.(2014): A New Species of *Azygia* (Trematoda: Azygiidae) from Pirate Perch, *Aphredoderus sayanus* (Aphredoderidae), in the Big Thicket National Preserve, Texas, U.S.A *Comparative Parasitology*, 81(2):257-259. 2014
- Bhalerao, G.D. (1942): On two helminth of *Mastacembelus pancalus*(Ham) *Rec. Ind. Mus. (44) 191-192*
- Bhure D.B., Nanware S.S. and Dhondge R.M. (2010): Record of the digenetic trematode *Azygia angusticauda* (Stafford,1904) Bhalerao,1942 from *Channa gachua* (Bloch.). *The Ecosphere (An International Biannual Journal of Environment and Biological Sciences)*. Vol.1(1) pp-1-3
- Dawes B.(1947): The Trematoda of British Fishes. *The Ray Society, London. 344 pp.*
- Gaikwad Kanchan Gulabrao, Nanware Sanjay Shamrao and Bhure Dhanraj Balbhim (2019): Studies on *Azygia gigantea* Sp. Nov. (Digenea : Azygiidae Odhner,1911) of Freshwater fish *Channa punctata* (Bloch). *Life Science Bulletin*. Vol. 16(1&2) pp 182-184
- Gibson, D. I. (2002): Chapter 4: Superfamily Azygioidea Luhe, 1909. Pages 19–24 in D. I. Gibson, A. Jones, and R. A. Bray, eds. *Keys to the Trematoda*, Vol. 1. CAB International and the Natural History Museum, London, U.K.
- Goldberger, Joseph, (1911): Some known and three new endoparasitic trematodes from American freshwater fish. *Bull. Hyg. Lab., 71: 7-35*
- Khillare, C. A., Lohiya V. N. and Khade R. N. (2022): Morphological investigation of a new species of *Azygia*, Diesing, 1854 from freshwater fish *Channa punctatus* (BLOCH) in Buldhana District (M.S.) India. *International Journal of Creative Research Thoughts (IJCRT)*. Volume 10, Issue2,pp337-341.
- Leidy, Joseph, (1851): Contributions to helminthology. *Proc. Acad. Nat. Sci. Philadelphia*, 5:205-210
- Looss, A (1899): “Weitere Beitrage Zur Kenntis der Trematoden fauna Aegyptens” *Zool. Jahrb. Syst*, 12: 521-784
- Looss, A. (1899): Weitere Beitrage zur kenntnis der Trematoden fauna Aegyptens ziglich Versuch einer naturlichen Gliederung des Genus *Distomum*. *Retzius Zool. Jahrb. Syst*. 12: 512-784
- Manter, H. W. (1926): Some North American fish trematodes III. *Biol. Monogr. (10) 1-138*
- Moravec, F. Sey, O. (1989): Some trematodes of freshwater fishes from North Vietnam with a list of recorded endohemintns by fish host. *Folia Parasitol 36 (3) 243-262*
- Sillman, E., (1953b): Morphology and life history studies on *Azygia longa* (Leidy, 1851) and *Azygia acuminata* Goldberger, 1911, with taxonomic considerations in the genus *Azygia* Looss, 1899 (Trematoda: Azygiidae). *Thesis: University of Michigan, Ann Arbor, Mich.*
- Stunkard, H. W (1956): The morphology and life history of digenetic trematode *Azygia* Sebago, Ward, 1910. *Biol.Bull. Woods Hole.(111): 248-268.*
- Van Cleave, H. J., and J. F. Mueller,(1934): Parasites of Oneida Lake fishes. III. A biological and ecological survey of the worm parasites. *Roosevelt Wildlife Annals*, 7: 161-334
- Ward, H. B. (1910): On the structure and classification of North American parasite worms. *J. Parasit. 4: 1- 11.*
- Wooton, D. M. (1957): Note on the life cycle of *Azygia acuminata* Goldberger, 1911 (Azygiidae-Trematoda). *Biol. Bull. (113) 488-498*