Morphology-Based Identification of Rotifers for Sustainable Aquaculture

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Abstract
Rotifers are microscopic zooplankton that play a crucial role as live feed in aquaculture, particularly during the early developmental stages of fish and crustaceans. Their small size, high nutritional value, ease of digestion, and rapid reproductive capacity make them an ideal food source for larvae, fry, and fingerlings. Accurate identification of rotifer species is essential for their effective utilization and for advancing ecological and taxonomic studies. Although molecular techniques such as DNA barcoding have gained importance, morphological identification remains a simple, cost-effective, and widely used approach. The present study focuses on the morphological classification and identification of rotifers belonging to the phylum Rotifera. Detailed taxonomic keys have been developed based on distinguishing morphological features including body shape, lorica structure, corona, mastax, and trophi types. The classification hierarchy comprises major groups including Monogononta and Bdelloidea, along with their respective orders, families, genera, and species. Identification keys are provided for several important taxa, including genera such as *Brachionus*, *Keratella*, *Lecane*, *Trichocerca*, and *Asplanchna*. This work serves as a comprehensive guide for the identification of rotifer species using morphological characteristics, which can facilitate taxonomic studies, biodiversity assessments, and aquaculture practices.

Keywords: Zooplankton, Aquaculture, *Brachionus*, Lorica, Trophi.

Introduction

Live feed known to play a very important role in hatcheries for the feeding of fish larvae, fry and fingerlings. Planktons are defined as tiny water drifters that cannot swim against water currents. These organisms of adequate size have been used as live food for feeding crustaceans and fish in intensive aquaculture (Pachiappan *et al*., 2018). Zooplanktons have been considered as essential feed for the growth of larvae, as they are easily ingested and digested (Kinne, 1997) and they do not affect the water quality (Watanabe *et al*.,1978). They are an important natural basic diet called "living capsule of nutrition", for many fish and shrimps, as they serve as an intermediary species in food chain that transfers energy from small planktonic algae to larger fish (Santos-Wisniewski *et al*., 2006).

The common live feeds in aquaculture include the members of rotifers (*Brachionus*), freshwater cladocerans (e.g*., Daphnia* and *Moina*) and copepods (*Mesocyclop*) (Kandathil *et al*., 2020). All these have been known for their high reproductive potential, ability to reproduce at a higher rate in a short period and capacity to survive in adverse conditions (Neelakantan *et al*., 1988). Rotifers offered many advantages over other zooplankton as live feed *viz*., small size, high digestibility with good absorption rate, availability in water body and high nutritional value (Lubzens *et al*., 1989). The size of marine fish larvae, mostly very small at the time of hatching, ranges from 2-7 mm except for a few species (Pauly and Pullin, 1988). Therefore, the feed provided to them must meet their requirements for better growth and survival. *Brachionus plicatilis* has served as the first food during the initial days of exogeneous feeding of larvae (Theilacker and Dorsey, 1980; Kissil, 1984). Various studies documented and recommended rotifers as important food for freshwater fish larvae and fry (Hale and Carlson, 1972; Siefert, 1972; Howell, 1973).

Many studies have been reported on various aspects of zooplankton *viz*., their ecological diversity, population dynamics, genome sequencing, and much more molecular work. To interpret and use these studies, a very important first step is the proper identification and nomenclatural determination of all zooplankton (Bakhtiyar *et al*., 2020). The organisms are classified either on the basis of morphological features or molecular markers. DNA barcoding is a widely used molecular technique for plankton identification. This technique uses short sequences of one or a few genes in order to classify known organisms and use this information to detect new ones (Antil *et al*., 2023). The morphological approach has been known as simple and less expensive than the molecular approach (Hillis, 1987). The morphological identification is one of the most common and useful approaches in this area, though DNA barcoding is another molecular based approach to identify and classify zooplankton. Based on this background, this study aims to find the morphological identification keys of rotifer species that help in the identification and classification of these organisms.

Classification of rotifers

Rotifera, a group of microscopic invertebrates, primarily freshwater, tiny, free-living zooplankton (Balian *et al*., 2008). The word "Rotifera" is derived from the Latin word, which means "wheel-bearer". This name is given on the basis of the crown of cilia present in the mouth region that appears as a wheel. The general characters of rotifers (Fig. 1) are as follows:

Transparent, elongated, relatively cylindrical, bilaterally symmetrical, and non-segmented body with pseudocoelom.

Epidermis has a fixed small number of nuclei, strengthened into a distinctive armor, called "Lorica".

Body divided into 3 parts; head, trunk and foot.

The head bears a crown of cilia often called "corona", the most distinctive feature of rotifera, organized as two-wheel like ciliary organ, from which the name of the group is derived.

The mouth ventral and open into pharynx, called the "mastax", the second important distinctive feature of the rotifers.

The mastax has powerful walls and contains small jaw-like structures called "trophi", varies from species to species, and helps to bite and chew the prey.

The trunk, the major part of the body, encloses most of the internal organs.

Foot reduced or totally absent in free-swimming species, projects from the posterior part of the trunk, and is usually narrow, appearing as a tail. It may retract partially or wholly into the trunk.

Respiratory and circulatory system organs are lacking.

Rotifers reproduce sexually as well as asexually. Parthenogenesis is the common way of reproduction among rotifers.

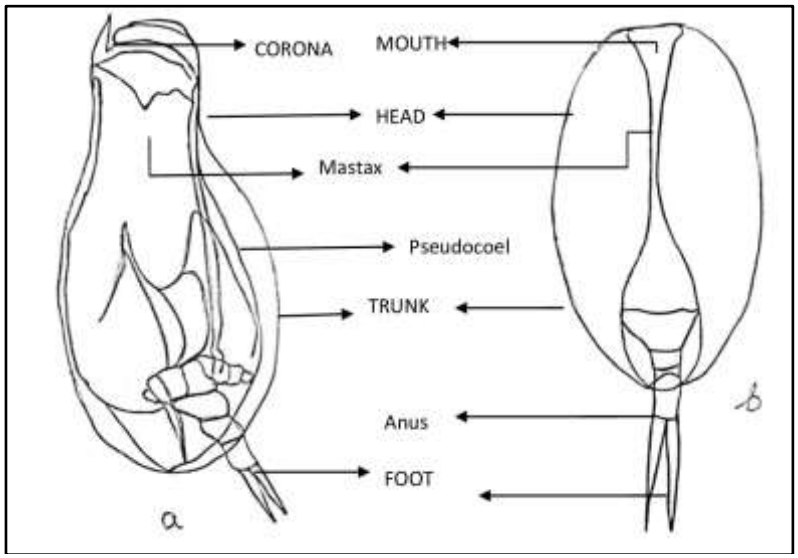


Fig.1. Morphology of the Rotifers; a. lateral view; b. ventral view.

Classification of Rotifera

Phylum Rotifera comprises 2030 species. The detailed classification of the phylum Rotifera, with some important species are given below. Their taxonomy is still in the state of flux. Rotifera are classified into two super-classes: Pararotatoria and Eurotatoria. Pararotatoria are the most primitive rotifers and are exclusively marine with only one family; seisonidae, 2 genera, and 3 species (*Paraseison annulatus, Seison nebaliae*, *Seison africanus*), not included here. Eurotatoria is further divided into two classes: monogononta with 1570 *spp*. and bdelloidea with 461 *spp*. (Glime 2008). Strictly speaking, rotifera phylum is confined to Monogononta and Bdelloidea.

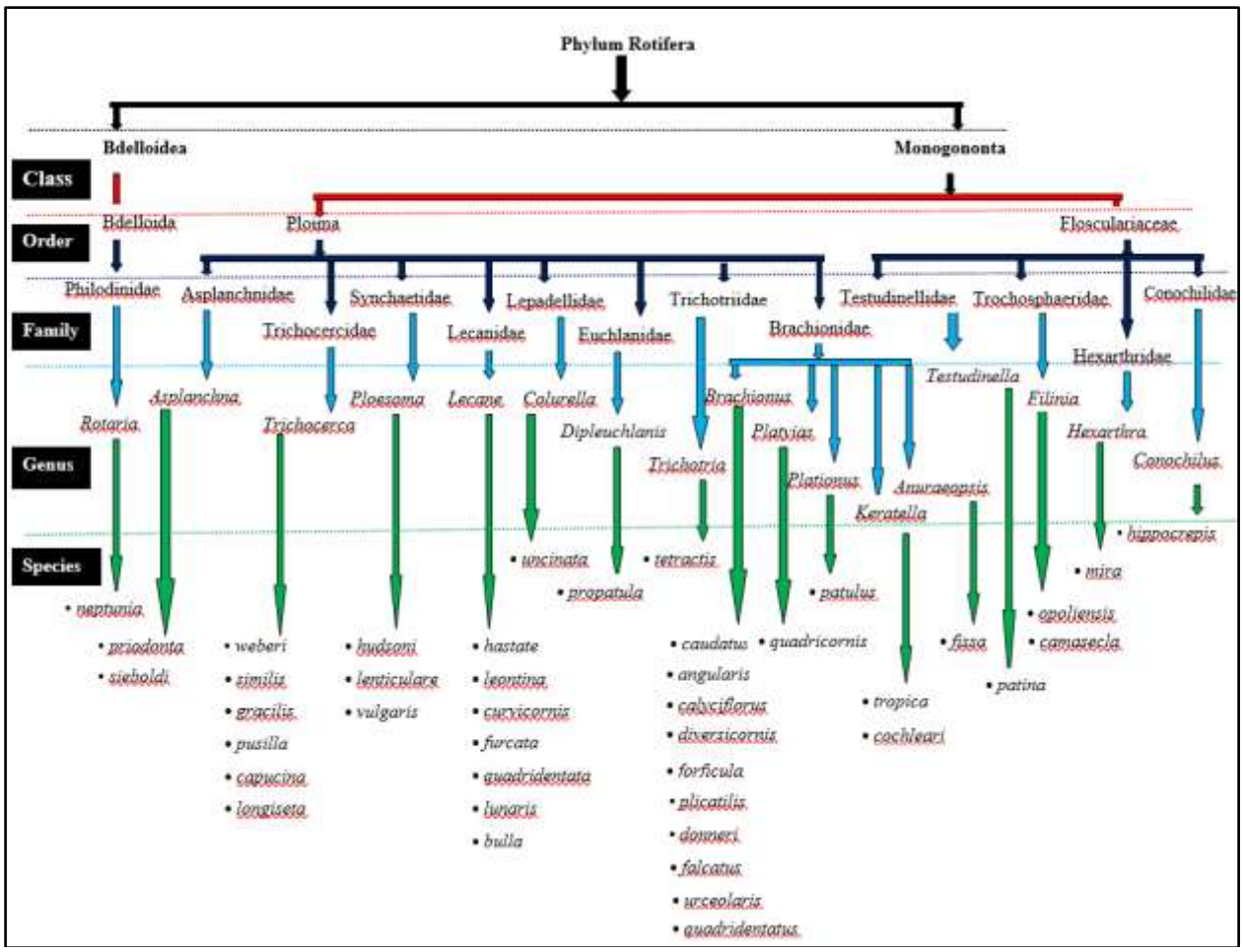


Fig.2. Classification of phylum Rotifera (Cuvier 1798).

Identification of Rotifers

Rotifers have unique features that varies species to species. These features can be used as an identifying key for particular individuals. Some specific identifying keys for different classes, orders, family, genus, and species have been discussed below.

Identifying the key to the Class of the Phylum Rotifera

- Two ovaries present: - Class Bdelloidea, Order Bdelloida, Family Philodinidae, Genus Rotaria (Fig. 3).
- One ovary present: - Class Monogononta.

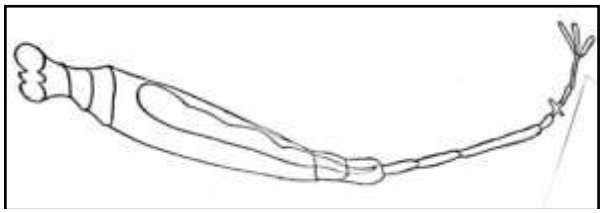


Fig. 3. *Rotaria neptunia*, lateral view.

Identifying key to the Order of the Class Monogononta

- Trophi types are forcipate, incudate, malleate, or virgate: - Order Ploima.

Trophi type is ramate, elliptical to round corona, horseshoe shaped or four-lobed, heart shaped, without long setae: - Order Flosculariaceae (Fig. 4).

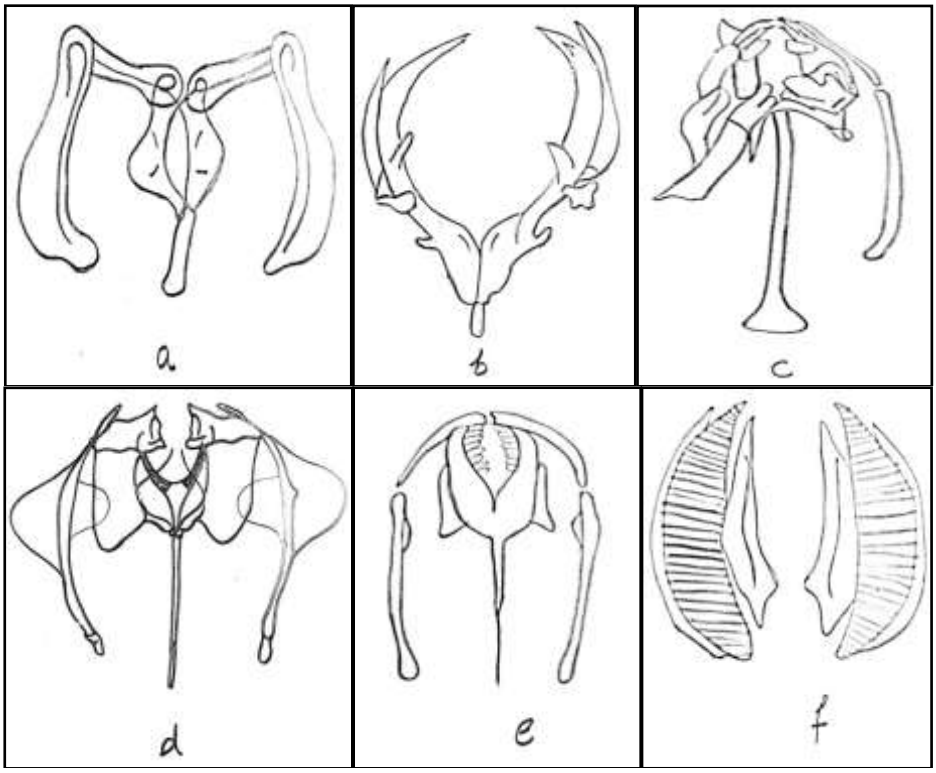
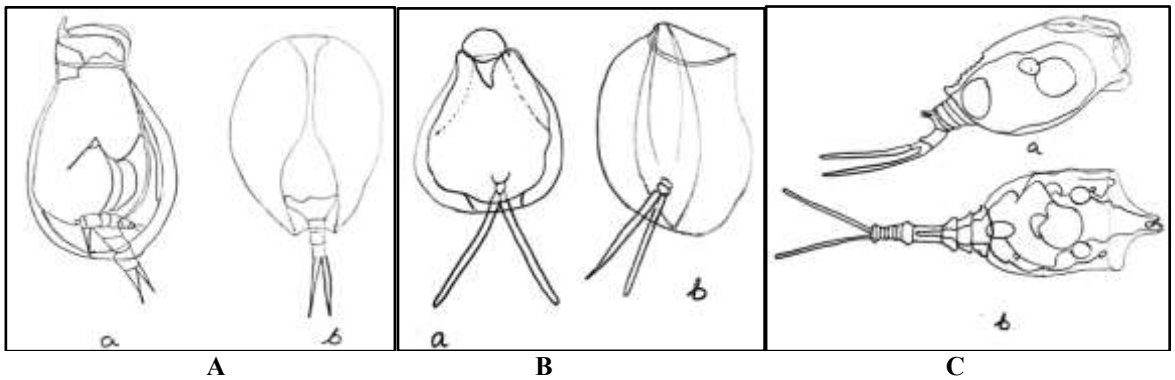


Fig.4. Rotifer trophi types; a. Malleate trophi; b. Incudate trophi; c, d. Virgate trophi; e. Forcipate trophi; f. Ramate trophi.

Identifying the key to the Families of Order Ploima

- Trophi incudate: - Family Asplanchnidae.
- More or less asymmetrical body. Asymmetrical trophi, asymmetry refers to all components of trophies. Terminal foot, short single pseudo-segment with many bristles and an elongated spine-like toe, unequal in length: - Family Trichocercidae.
- Trophi symmetrical, toes missing or present in similar length, single toe, rarely. Body shape, sacciform, fusiform, conical, never with dorsal crest: - Family Synchaetidae.
- Decreased number of unci teeth, normally composed of 3 stout subequal fused teeth. Foot short, a single pseudo-segment inserted ventrally on the ventral plate. Toes 2 or fused partially or entirely to 1. Usually loricate with dorsal and ventral plates separated by lateral furrows (sulci): - Family Lecanidae.
- Head shield present, retractile or non-retractile: - Family Lepadellidae (Fig. 5A).
- Two stout toes on foot, lateral sulci present: - Family Euchlanidae (Fig. 5B).
- Defined head, trunk and foot, covered by lorica: - Family Trichotriidae (Fig. 5C).
- Only trunk, occasionally foot if present, covered by lorica: - Family Brachionidae.

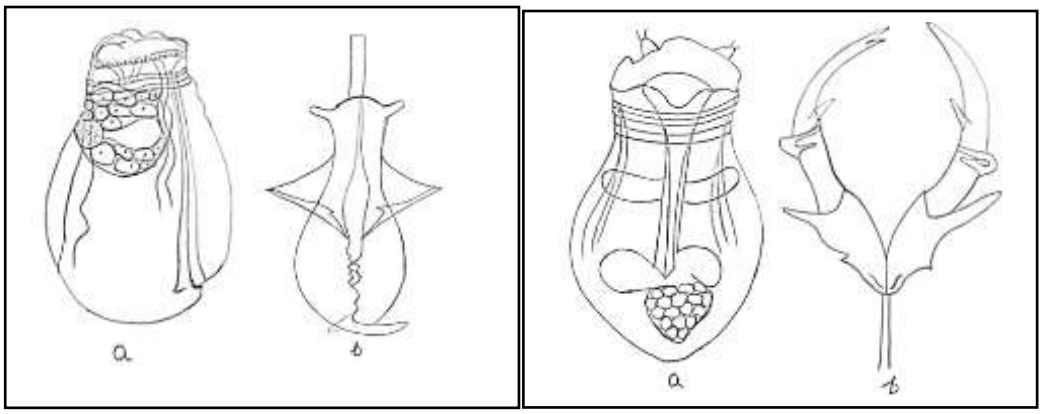


A **B** **C**

Fig. 5. A. *Colurella uncinata*; B. *Dipleuchlanis propatula*; C. *Trichotria tetractis*; a. lateral; b. dorsal view.

Identifying the key to the species of genus *Asplanchna*

Sacciform or rounded lorica, distal part of ramus with four to six teeth on inner margin: - *Asplanchna priodonta* (Fig. 6A). Lorica elongated or horse-shoe shaped: - *Asplanchna sieboldi* (Fig. 6B).

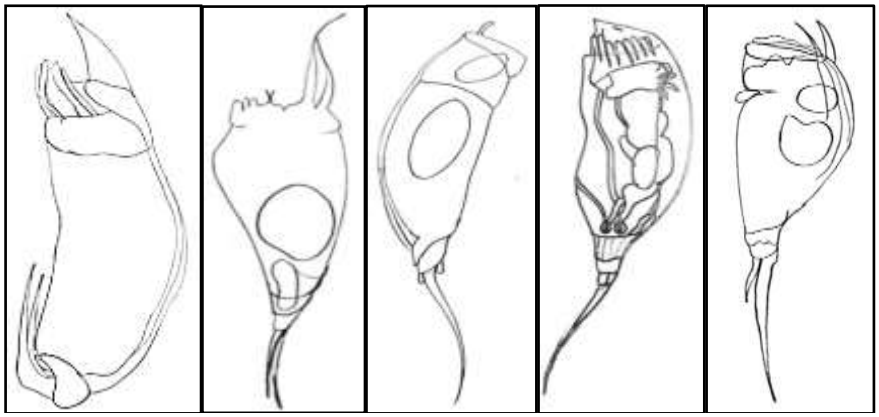


A B

Fig.6. A. *Asplanchna priodonta*; B. *Asplanchna sieboldi*, a. lateral view (Female); b. trophi.

Identifying the key to the species of Genus *Trichocerca*

- One spine in anterior margin, lateral plate present: - *Trichocerca weberi* (Fig. 7A).
- Absence of lateral plate, anterior margin of lorica with two spines, double keel, foot not projected over by posterior margin: - *Trichocerca similis* (Fig. 7B).
- No spines, margin of lorica with blunt projections: - *Trichocerca pusilla* (Fig. 7C).
- Two spines present, dorsally cowl-like structure: - *Trichocerca capucina* (Fig. 7D).
- Two spines of unequal length, no overhang, body not constricted, single crook present in left manubrium: - *Trichocerca longiseta* (Fig. 7E).

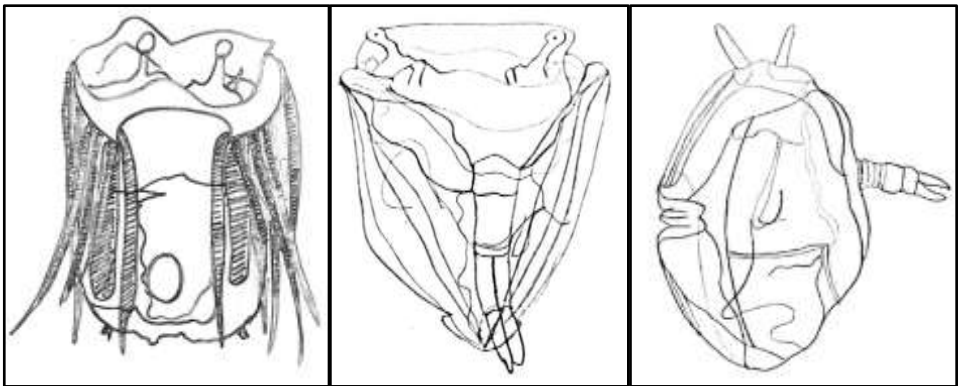


A B C D E

Fig.7. A. *Trichocerca weberi*; B. *Trichocerca similis*; C. *Trichocerca pusilla*; D. *Trichocerca capucina*; E. *Trichocerca longiseta*.

Identifying the key to the Genera of Family Synchaetidae

- Illoricate body, dorsal part of the body square or somewhat rectangular, dorso-ventrally compressed. At the anterior dorsal and ventral corners, four bundles of serrated blade-shaped appendages are present: Genus *Polyarthra* (Fig. 8A).
- Loricate body with head shield ornamented with grooves and ridges, and general reticulation sometimes. Lorica presents with a foot aperture or ventral fissure. Long, flexible foot present with two toes: Genus *Ploesoma.*
- Ribbed lorica, protuberance at anterior margin: - *Ploesoma lenticulare* (Fig. 8B).
- Smooth lorica surface: - *Ploesoma hudsoni* (Fig. 8C).

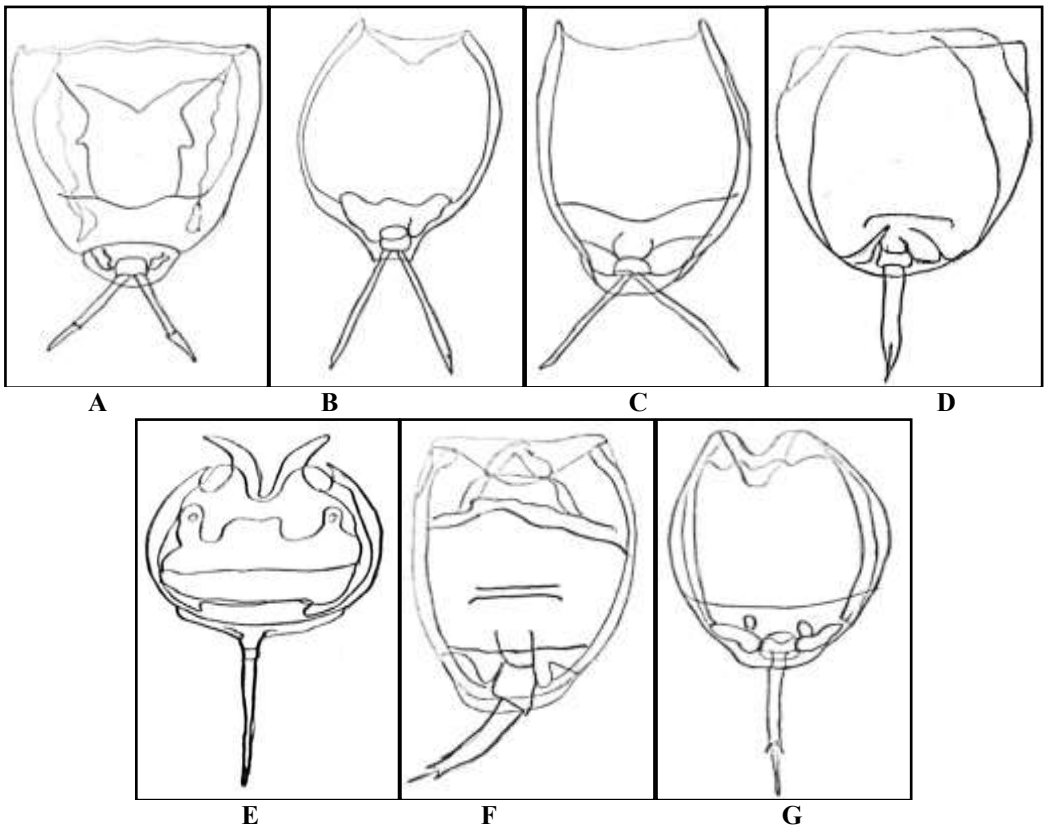


A B C

Fig.8. A. *Polyarthra vulgaris*, dorsal view; B. *Ploesoma hudsoni*, ventral view; C. *Ploesoma lenticulare*, lateral view

Identifying the key to the species of Genus *Lecane*

- Knob like swelling in claw: - *Lecane hastata* (Fig. 9A).
- Elongated footplate in ventral plate: - *Lecane leontina* (Fig. 9B).
- Symmetrical and rounded ventral caudal margin, unornamented surface, concave dorsal anterior margin: - *Lecane curvicorni* (Fig. 9C).
- Claw absent in toe, more or less large fontal corner cusps/spines in anterior margin of lorica, ventral lorica plate not constricted: - *Lecane furcata* (Fig. 9D).
- Median curved spine in anterior margin of lorica: *Lecane quadridentata* (Fig. 9E).
- Anterior margin of lorica deeply sinuated, ventral sinus deeper than dorsal, rounded posterior margin: - *Lecane lunaris* (Fig. 9F).
- Lorica margin somewhat wide, sinuated, concave dorsal and ventral anterior margins, head aperture narrow to taper: - *Lecane bulla* (Fig. 9G).



A B C D

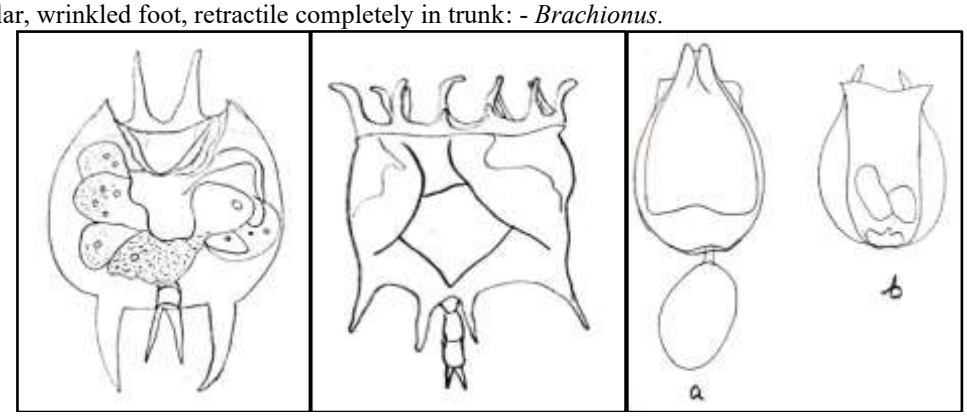
E F G

Fig.9. A. *Lecane hastata*; B. *Lecane leontina*; C. *Lecane curvicorni*; D. *Lecane furcata*; E. *Lecane quadridentata*; F. *Lecane lunaris*; G. *Lecane bulla*, ventral view.

Identifying the key to the Genera of Family Brachionidae

- Lorica flattened dorso-ventrally, opening of foot on ventral: - *Platyias* (Fig. 10A).
- Lorica high in cross-section, opening of foot on terminal: - *Plationus* (Fig. 10B).

- Spines absent in lorica: - *Anuraeopsis* (Fig. 10C).
- Spines in lorica: - *Keratella.*
- Tubular, wrinkled foot, retractile completely in trunk: - *Brachionus.*

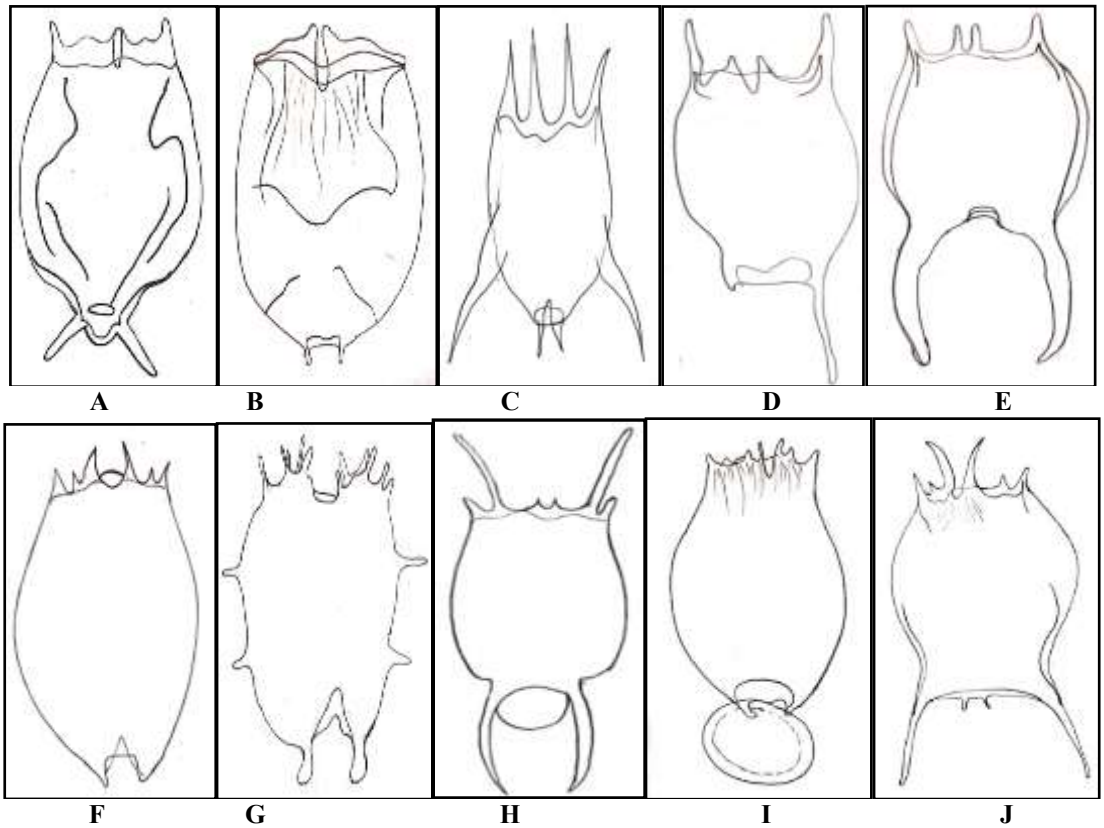


A **B** **C**

Fig.10. A. *Platyias quadricornis*; B. *Plationus patulus*; C. *Anuraeopsis fissa*, a, dorsal view with egg; b, ventral view

Identifying the key to the species of Genus *Brachionus*

- Two divergent posterior spines present: - *Brachionus caudatus* (Fig. 11A).
- Absence of posterior spines: - *Brachionus angularis* (Fig. 11B).
- Lorica smooth, transparent, posteriorly circular: - *Brachionus calyciflorus* (Fig. 11C).
- Unequal spines in posterior lorica, right spine longer: - *Brachionus diversicornis* (Fig. 11D).
- Fewer equal spines in posterior lorica, curved inwardly: -*Brachionus forficula* (Fig. 11E).
- Absence of spines in posterior lorica, halophilic species: - *Brachionus plicatilis* (Fig. 11F).
- Presence of two large spines in posterior lorica: - *Brachionus donneri* (Fig. 11G).
- Antero-median and antero-lateral spines are smaller than antero- intermediate spines, posterior two long spines are present: - *Brachionus falcatus* (Fig. 11H).
- No spines in the lateral posterior side: - *Brachionus urceolaris* (Fig. 11I).
- Lateral posterior side with spines, anterior median spine longer and curved than other spines: - *Brachionus quadridentatus* (Fig. 11J).



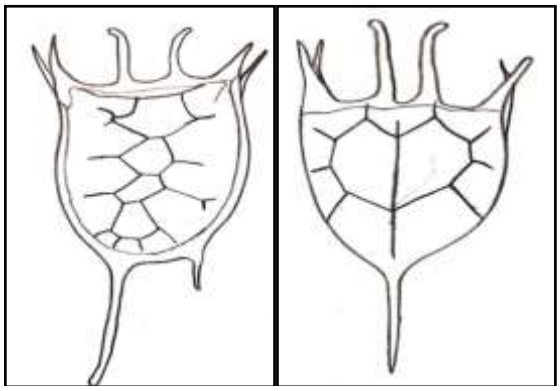
A **B** **C** **D** **E**

F **G** **H** **I** **J**

Fig.11. A. *Brachionus caudatus***;** B. *Brachionus angularis*; C. *Brachionus calyciflorus*; D. *Brachionus diversicornis*; E. *Brachionus forficula*; F. *Brachionus plicatilis*; G. *Brachionus donneri*; H. *Brachionus falcatus*; I. *Brachionus urceolaris*; J. *Brachionus quadridentatus*.

Identifying the key to the species of Genus *Keratella*

- Two spines in the posterior margin of the lorica, one line in the dorsal plate with five pieces: - *Keratella tropica* (Fig. 12A).
- One spine in the posterior margin of the lorica, median line in the dorsal plate extending longitudinally behind the median frontal area to the base of the posterior spine: - *Keratella cochlearis* (Fig. 12B).



A **B**

Fig.12. A. *Keratella tropica*, ventral view; B. *Keratella cochlearis*, dorsal view.

Identifying the key to the Family of Order Floscullariaceae

- Presence of six arm-like appendages in the body: - Family Hexarthridae (Fig. 13A).
- Presence of foot: - Family Conochilidae (Fig. 13B).
- Loricate body: - Family Testudinellidae (Fig. 13C).
- Absence of foot: - Family Trochosphaeridae, genus *Filinia*.

Identifying the key to the species of Genus *Filinia*

- Presence of two unequal setae in caudal region: - *Filinia opoliensis* (Fig. 13D).
- Presence of one seta in caudal region: - *Filinia camasecla* (Fig. 13E).

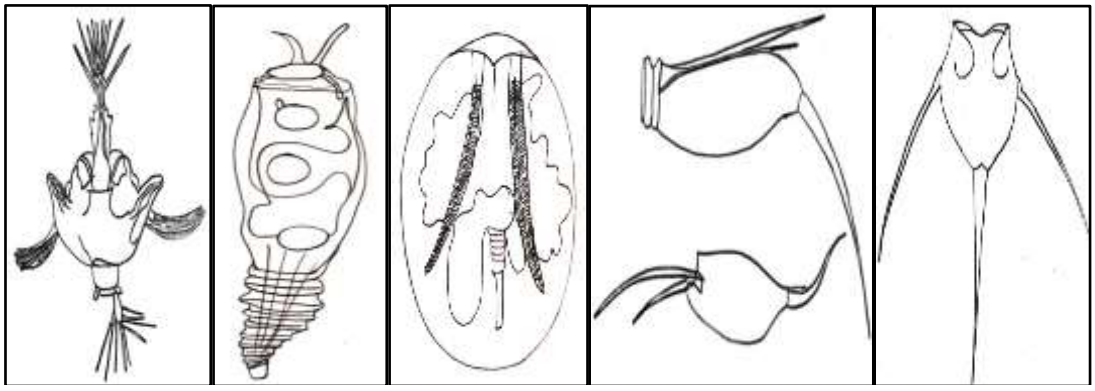


Fig.13. A. *Hexarthra mira*, ventral view; B. *Conochilus hippocrepis*; C. *Testudinella patina*, ventral view; D. *Filinia opoliensis*, lateral view; E. *Filinia camasecla,* lateral view.

Conclusion

Rotifers are an essential component of aquaculture due to their high nutritional value and suitability as live feed for fish larvae and other aquatic organisms. This study emphasizes the importance of accurate identification of rotifer species using morphological characteristics. The detailed identification keys provided at various taxonomic levels, from class to species, offer a practical and cost-effective approach for distinguishing different rotifer taxa. Despite the growing use of molecular techniques, morphological identification remains highly relevant, especially in routine and field-based studies. The findings of this work can support aquaculture practices, biodiversity assessments, and further taxonomic research, while also serving as a useful reference for researchers and students in the field of aquatic biology.

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