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AMMONIUM PICRATE GLYCERINE AND SODIUM DODECYL SULPHATE AS A RAPID CLEARING AGENT FOR MORPHOLOGICAL STUDIES OF *DACTYLOGYRUS*

The genus *Dactylogyrus* is the largest helminth genus with more than 900 species (Neary *et al*., 2012). It represents the most dominant genus among the monogenea with regards to host distribution and location. These are common ectoparasites living in gills of freshwater fishes (Woo, 2006). They seem to be an appropriate model for studying the process of parasite diversification, mainly because of their high species richness and, morphological and ecological diversity. They are attached to the host surface by a characteristic opisthaptor which is species-specific and has anchors, hooks and hooklets (order: Monopisthocotylea). These are hermaphroditic flatworms of aquatic vertebrates.

Species identification of monogeneans is based mainly on the morphology of the hard parts of their haptors and copulatory organs (Bruno *et al*., 2006). Previously ammonium picrate glycerine (APG) has been widely used for fixing and clearing monogenean specimens in order to study the sclerotized parts using phase-contrast microscopy (Lim and Furtado, 1986). Also selective staining methods have been developed to investigate the morphology of the sclerites of monogeneans (Zdarska, 1976; Kritsky *et al*., 1978; Richards and Chubb, 1995); but these techniques are time consuming and again the hard parts are not clearly defined. To overcome these problems sodium dodecyl sulphate (SDS) (5-10%) along with 1 M NaOH was used as an alternative for clear visualisation of hard parts of the monogeneans (Wong *et al*., 2006). Though this technique is well established and renders good morphological results; still an attempt has been made here by using a mixture of the above two reagents to study the morphology of monogenean *Dactylogyrus*. SDS is an anionic detergent that denatures and solubilizes the biological membrane; and APG fixes as well as furnishes a yellow stain to the worm that provides a vivid, distinct and attractive image of the parasite for its identification.

Adult speciemens of *Dactylogyrus* sp. collected from infected *Catla catla* from Andhra Pradesh was used in this study. Ammonium picrate glycerine and 10% SDS mixed together in the ratio of 1:1 were used on alcohol (70%) fixed specimens. The specimens were rehydrated through a series of decreasing concentrations of ethanol to water prior to APG and SDS mixture treatment. The worms were then placed in a small drop of water on a slide and a drop of the prepared mixture was added before flattening the parasite with a cover slip. Excess solution on the edge of the cover slip was drained off with a filter paper and sealed using nail polish to prevent the specimens from drying out and to secure the parasite in position. The treated specimens were left for 10 minutes

before viewing them under the phase contrast microscope. A total of 50 specimens were fixed on slides and then observed under the Nikon (Japan) Eclipse 50i phase contrast microscope, 0.90 dry.

Photomicrograph images of these treated specimens were taken and compared. The tissues of monogenean specimens fixed in ammonium picrate glycerine appeared opaque and granulated, 10% SDS with 1 M NaOH only confined to the hard parts while their mixture clearly revealed the whole morphology and better resolution of all body parts of the flatworm (Fig. 1C) as compared to the other two (Figs. 1A & 1B). The marginal hooks, anchor and bars of *Dactylogyrus* sp. were also clearly visible with the mixture of APG and SDS solution (Fig. 2C) as compared to the other two reagents (Figs. 2A & 2B). Even this mixture clearly revealed copulatory organs (Fig. 3C) as compared to use of any of these reagents alone (Fig. 3A & 3B).

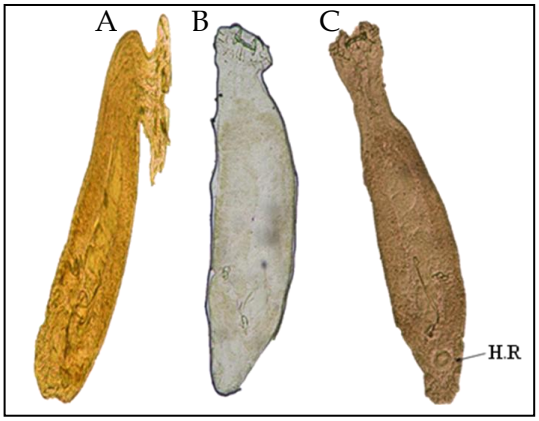

Fig. 1. Phase-contrast microscope images of whole body morphology of *Dactylogyrus* sp. mounted in (A) Ammonum picrate glycerine (APG), (B) Sodium dodecyl sulphate (SDS) in 1M NaOH and (C) APG + 10% SDS. H.R, Haptor region.

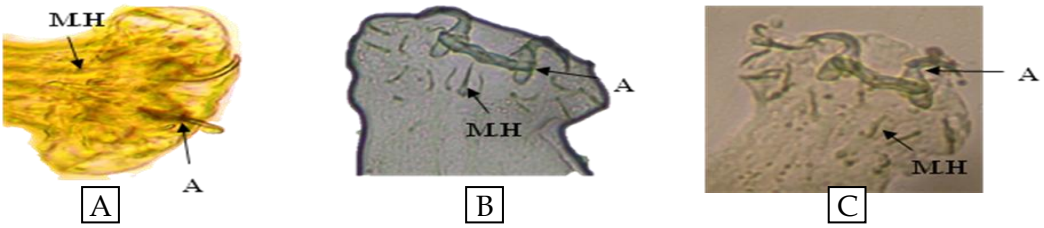

Fig. 2. Opisthaptor of *Dactylogyrus* sp. mounted in ammonium picrate glycerine (A), SDS (B) and ammonium picrate glycerine and SDS together (C). A, Anchor; M.H, Marginal hook.

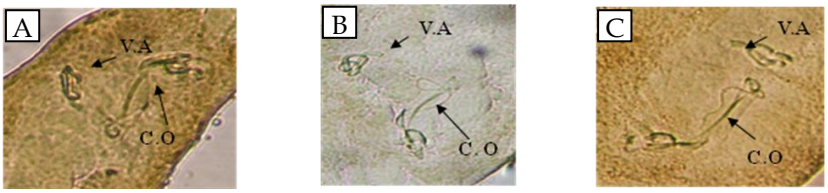

Fig. 3. Copulatory organ of *Dactylogyrus* sp. fixed in ammonium picrate glycerine (A), SDS (B) and in ammonium picrate glycerine and SDS (C). C.O, Copulatory organ; V.A, Vaginal apparatus

Ammonium picrate glycerine and 10% SDS treated specimen could be kept safely for more than 6 months under laboratory conditions. The present study shows that ammonium picrate glycerine and 10% SDS together can be used as a rapid clearing agent for transparency besides staining, thereby revealing the morphology for taxonomic identification to species level. The technique may be useful for better taxonomic description and identification of other parasites with hard structures as well as for those parasites whose identification is curtailed due to limited resolution of morphological features.

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REFERENCES

Bruno, D. W., B. Nowak and D. G. Elliott, 2006. Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. *Dis. Aquat. Org*., **70**: 1-36.

Kritsky, D. C., P. D. Leiby and R. J. Kayton, 1978. A rapid stain technique for haptoral bars of *Gyrodactylus* species (Monogenea). *J. Parasitol.,* **69**: 862–865.

Lim, L. H. S. and J. I. Furtado, 1986. Sixteen new species of *Dactylogyrus* from the genus *Puntius* Hamilton (Cyprinidae). *Fol. Parasitol.,* **33**: 21–34.

Neary, E. T., N. Develi and G. Ozgul, 2012. Occurrence of *Dactylogyrus* species (Platyhelminths, Monogenean) on cyprinids in Almus Dam Lake, Turkey. *Turkish J. Fish. Aquatic Sci.,* **12**: 15-21.

Richards, G. R. and J. C. Chubb, 1995. Trichrome staining of *Gyrodactylus* sclerites and soft tissues following fixation in ammonium picrate-glycerin, including an improved rendition of the haptoral bars of *G. turnbulli*. *J. Helminthol.,* **69**: 149–154.

Wong, W. L., W. B. Tan and L. H. S. Lim, 2006. Sodium dodecyl sulphate as a rapid clearing agent for studying the hard parts of monogeneans and nematodes. *J. Helminthol.,* **80**: 87–90.

Woo, P. T. K., 2006. Fish diseases and disorders: protozoan and metazoan infections. CAB International, London, 791 pp.

Zdarska, Z., 1976. Selective staining of opisthaptor sclerites of some monogeneans. *Fol. Parasitol.,* **23**: 182.

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