PANAMJAS
Pan-American Journal of Aquatic Sciences

A new proposal for the optimization of morphological analyses of micro and macroinvertebrates in ecological freshwater studies

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Abstract The study of specimens in ecological freshwater studies needs the preparation of a large number of microscope slides, with effort of economic resources and space for storage. A modification in the mounting technique and storing for insect larvae and microcrustaceans is presented.

Key words: Larvae; aquatic Arthropoda; Mounting Technique.

Resumo. Uma nova proposta para a otimização do estudo morfológico de micro e macroinvertebrados em pesquisas ecologiças de água doce. O estudo de espécimes em estudos ecológicos de água doce prevê a preparação de uma grande quantidade de lâminas, com gastos de recursos econômicos e de espaço para estocagem. Uma adaptação na técnica de montagem para larvas de insetos e microcrustáceos é apresentada.

Palavras-Chave: Larva; Arthropoda aquáticos; Técnica de montagem.

In freshwater ecology, identification and morphological study of macroinvertebrate is fundamental. In this kind of study, in general thousands of specimens must be analyzed and successively stored in a collection as testimonial of the research (Callisto et *al*. 1998, Mugnai & Gatti 2008). Beside, the main problems in this kind of studies are time consuming for identification, material costs and space utilized for storing the specimens.

To identify and stored material from a hyporheic fauna research collected in streams of *Parque Nacional da Tijuca* in Rio de Janeiro City, State of Rio de Janeiro, Brazil, it was decided to use some alternative techniques and this work turns to a proposal that tries to mitigate the aforementioned difficulties for limnological and ecological studies and collections.

Freshwater invertebrate are technically subdivided, based on the larval or imaginal size, in macroinvertebrate, up to 1mm, and microinvertebrate, less to 1mm (Tachet et *al*. 1987).

Most of freshwater macroinvertebrate are insect larvae, especially Chironomidae that can be dominant, especially in impacted areas (Armitage et al. 1995, Mugnai et al. 2008). Members of the family Chironomidae occupy a wide range of habitats from aquatic to terrestrial. More than 4,000 species belonging to 339 genera and 11 subfamilies are aquatic in the immature stage (Ferrington 2008). This family is the most widely distributed and frequently the most abundant aquatic insects reaching densities of 50,000 per square meter (Coffman 1996).

The Chironomidae are important in limnological studies. The larvae of Chironomidae explore a wide variety of habitats and have a wide variety of types of food and eating behaviors (Armitage *et al*. 1995). In freshwater environments, the larvae colonize basically the sediment and

aquatic vegetation, showing wide range of conditions under which they can live.

Are potential biomarkers, with some species restricted in good quality water; otherwise, the incidence of deformities of the mandible has been used for assessing pollution (Coffman 1996).

Unfortunately, even today the larval form of many species remains not identified and some are difficult to identify (Epler 2001, Trivino-Strixino 2011).

Prepare and mounting larval chironomids specimens can be complex and time consuming. The most useful characters for identification are located in the ventral portion of the head capsule and in the antennae as mentum, pecten epipharyngis and flagellum. The examination of the characters in the last instars may be necessary.

The larva is mounted on microscope slides using as mounting medium CMC-10, Hoyer's, Canada balsam, CMCP 9/9AF or Euparal to build semi-permanent or permanent slides, each media with advantages and disadvantages (Schlee 1966, Beckett & Lewis 1982, Epler 2001, Trivino-Strixino 2011).

For mounting the larvae were decided to use, instead using microscope slide and coverslip, two coverslips, 24x32 mm size. The larva was mounted on a coverslip using a drop of Euparal and a second coverslip was used to cover the specimen (Fig. 1A). To conserve the specimen and prevent fungi attack the coverslips border was sealed with Euparal instead transparent nail lack like use in other collection to seal microscope slides that being more time resistant (S. Taiti *pers. com*.).

To avoid accidental breakage during the study to store the specimens, a temporary slide storage boxes was building. The bottom of a box was covered with an Ethylene vinyl acetate (EVA) sheet with 2 mm thickness. Parallel cuts were made in EVA with a scalpel at a distance of 1 cm to allow housing the coverslips vertically (Fig. 1B). For final storing the coverslips can placed in the original coverslip commercial boxes separated by paper (Fig. 1C) or in microscope slides boxes, storing two specimens each slide, separated by a folded paper reporting the number of collection (Fig. 2).

The proposed strategy is efficient. To view under microscope, the larvae specimens mounted in coverslip-coverslip are placed over a microscopy slide (Fig. 1D). The thickness of the coverslip-coverslip does not obstruct the viewing and allows seeing the both sides of specimen when needed. That can be particularly useful in the study of small and medium larvae size avoiding bleaching using KOH.

Figure 1: **A,** larva was mounted on a coverslip-coverslip; **B,** study storage box; **C,** viewing under microscope; **D,** final storing in commercial box

Figure 2: microscope slides boxes with folded paper and specimens.

The box with EVA and microscope slides boxes with folded paper allowing safe transport, and handling the specimen avoiding breakage. Permanent storage in slide boxes reducing 50% space in the collection respect microscope slide preparation. Permanent storage in commercial coverslip box allows reducing space more than 95%. For economic standpoint this technique have a cost 25% less respect the traditional technique.

In our work 779 specimens were prepared, identified and stored in seventeen original coverslips boxes, occupying a volume less than 16 cm x 8 cm x 4 cm. All specimens are deposited at the Entomological Collection of Museu Nacional/UFRJ.

This methodology was applied to optimize the work with a large number of specimens of Chironomidae larvae and untested with other orders or families, but it is quite possible that procedure is applicable at other taxonomical group in the same stages of development for specimen not too large and not involving very rigid exoskeleton.

The microinvertebrates are represented especially for Nemata, Copepoda, Cladocera, Ostracoda and Rotifera. For the morphological study under optical microscope several techniques was proposed: viewing the specimen using a microscope slide with a single cavity and coverslip by positioning the specimen on the edge (Graeter 1910); construction of temporary slide surrounding the specimens with pieces of broken coverslips (Krantz & Walter 2009); construction of temporary slide with plumbing rings (Boxshall & Halsey 2004); using slides with glycerol drops and viewing the specimen flipping the slide (Humes & Gooding 1964). Some creates obvious problems as biosegurity with coverslips breakage activity, or time consuming problem wherein is necessary to remove the cover slip for change the specimen position to views other anatomical parts.

To overcome these problems in microinvertebrates studies we have done a slide with a space to accommodate the specimen utilizing a microscope slide and four coverslips of 18x18 mm size. Coverslips were placed in the position as shown in Figure 3 and glued. To glue the slide and coverslips blade stained veneer Verniz Vitral incolor Acrilex® was used.

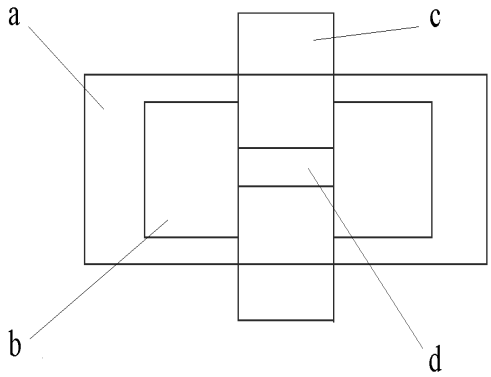

Figure 3. Microscope slide mounting scheme: a, microscope slide; b, coverslip; c, part of the coverslip to be broken; d, free space.

For an easy break along the edge of the slide of the coverslips that overcomes the edge of the slide is sufficient to brush a thick layer of glue on the lower surface of the coverslips. After a period of drying, depending on temperature, the coverslips can be broken with the hands and polished the edges with tiny sandpaper. The glue excess in the space that will host the specimen and between the coverslips should be removed with scalpel.

Before positioning the specimen is necessary to fill the space with a dense fluid, that can be glycerol or lactophenol. After placing the specimen, the slide is covered with a coverslip of 18x18 mm size or larger.

Using this technique enables, in many cases, repositioning the specimen directly under the optical microscope by simply laterally pressing and sliding the upper coverslip using a erasing pencil. For removal of the specimen is sufficient to pour a few drops of alcohol around the coverslip that it gently drop out. After using the slide, that can be washed and reused.

This type of microscope slide allows rapid repositioning of the specimen by rotating it about the longitudinal axis, allowing rapid visualization of all characters, that not need dissection, and perform photographic documentation.

As suspension fluids were tested lactophenol and glycerol. The first has a higher viscosity and is easier to use, but due to its hydrophilicity, dry or attract water, allows the use of the slide only for a limited period of time. The second allows to use the slide for a longer time, but, being more fluid the slide preparation can be a little more difficult.

Verniz Vitral incolor Acrilex® used to fix slide and coverslips is resistant to alcohol, water and other chemicals and has been tested to done permanent slides for microscopical studies and have a good cost benefit (Paiva et *al*. 2006).

This methodology was applied to optimize the studies activity with Hydracarina, Bathynellacea, Cyclopoda and Nemata. In addition, tests with Chironomidae were done. In this case the specimen's rotation is not possible, due to the size and curvature of the body, but provides temporary slides without damaging the specimen as happens with mounting coverglass on slide with glycerol. It is quite possible that this procedure is applicable at other taxonomical group with similar morphology and size.

Acknowledgements

We thank CAPES and FAPERJ for the financial support. Many thanks to Dr. Adriano Kury, Museu Nacional/UFRJ, for allow the research in the Arachnology Laboratory and Mario Jorge Gatti, Curator of Coleção Micológica de Trichocomaceae, IOC/FIOCRUZ, for the technical support.

References

Armitage, P. D., Cranston, P. S. & Pinder L. C. V. 1995. **Chironomidae: The Biology and Ecology of Non-Biting Midges**. London, Chapman & Hall Editora, 572p.

Beckett, D. C. & Lewis, P. A. 1982. An Efficient Procedure for Slide Mounting of Larval Chironomids. **Transactions of the American Microscopical Society**, 101(1): 96-99.

Boxshall, G. A. & Halsey, S. H. 2004. **An Introduction to Copepod Diversity**. The Ray Society, London, 966 pp

Callisto, M., Barbosa, F. A. R. & Vianna, J. A. 1998. Qual a importancia de uma coleçao cientifica de organismos aquaticos em um projeto de biodiversidade? An. **IV simpósio de ecossistemas brasileiros** 2: 432-439.

Coffman, W. P. 1996. Chironomidae. p. 345-376. *In:* Merrit, R.W. & Cummins K.W. **Aquatic insect of North América**. 3 th ed. Dubuque, Kendal/Hunt Publishing Company Editor, 497p.

Graeter, E. 1910. Die Copepoden der unterirdischer Gewässer. **Inaugural-Dissertation zur Erlangung der Doktorwürde, Universität Basel**. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart. 87 pp. + Plates I-III.

Epler, J. H. **Identification manual for the larval Chironomidae (Diptera) of North and South Carolina.** North Carolina, North Carolina Dep. Envir. Natural Resources, Div. Water Quality. 2001. Available online at: http://www.esb.enr.state.nc.us/BAUwww/Chironomid.htm [Accessed: 21/06/2012].

Ferrington, L. C. 2008. Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. **Hydrobiologia**, 595(1): 447-455.

Humes, A. G. & Gooding, R. U. 1964. A method for studying the external anatomy of copepods. **Crustaceana**, 6: 238-240.

Krantz, G. W. & Walter, D. E. (EDS.). 2009. **A Manual of Acarology**. Third Edition. Texas Tech University Press; Lubbock, Texas, 807 pp, ISBN 978-0-89672-620-8. $175.00.

Mugnai, R. & Gatti, M. 2008. Infra estrutura básica de suporte para o estudo de ecossistemas aquáticos. **Oecologia Brasiliensis**, 12 (3): 506- 519.

Mugnai, R., Oliveira, R.B., do Lago Carvalho, A. & Baptista, D.F. 2008. Adaptation of the Indice Biotico Esteso (IBE) for water quality assessment in rivers of Serra do Mar, Rio de Janeiro State, Brazil. **Tropical Zoology**, 21: 57-74.

Paiva, G. J. A. De, Fank-de-carvalho, S. M., & Pimenta, M. 2006. Verniz vitral incolor 500®: uma alternativa de meio de montagem economicamente viável. **Acta Botanica Brasilica**, 20(2): 257–264.

Schlee, D. 1966. Präparation und Ermittlung von Messwerten an Chironomidae (Diptera). **Gewässer und abwässer**, 41/42: 169-193.

Tachet, H., Bounard, M. & Richoux, P. **Introduction á l´étude des Macroinvértebrés des eaux douces**. Paris : C.R.D.P., 1987.

Trivino-Strixino, S. 2011. **Larvas de Chironomidae.** Guia de identificação. São Carlos, EdUFSCar Editora, 371p.

Received October 2014
Accepted February 2015
Published online April 2015