The family Chironomidae is the most ubiquitous and usually most abundant insect group in all types of freshwater often attaining larval population densities of many thousands per square meter. Their distribution extends to the limits of land, both north and south; indeed they are the dominant family in the Arctic region. Many species, mostly belonging to the subfamily Orthocladiinae, are terrestrial or semi-terrestrial and some are marine. Larvae of a species of Pontomyia, for example, have been found in the Atlantic Ocean to a depth of 30 m (Bretschko 1982).

It is not surprising then that the Chironomidae have attracted a great deal of scientific interest. The biology and ecology of the family is reviewed by Oliver (1971) and Armitage et al. (1995). Lists of all described chironomid species of the subfamilies Buchonomyiinae to Orthocladiinae are compiled by Ashe & O’Connor (2009, 2012), a third volume covering Chironomininae is in preparation. Bibliographies compiled since the beginning of the 1970s have resulted in a database containing almost 28,000 publications (Aagaard et al. 2012). The Chironomid homepage (http://insects.umzz.ums.lsa.umich.edu/~ethanbr/chiro/index.html) give, among other details, an updated list of Chironomid workers. The Chironomus Newsletter of Chironomidae Research (http://www.ntnu.no/ojs/index.php) annually publishes information on ongoing research. An international symposium on chironomids is held every 3rd year in a different part of the world.

Despite the attention which the family has received from specialists, in general ecological works the Chironomidae often are ignored or briefly mentioned. Some general faunistic studies of freshwater habitats may produce reasonable species lists for the family but detailed autecological studies remain few. The primary reason for these omissions is the difficulty with which the non-specialist is faced in identifying, especially larvae, to species, or in some cases even to genus. Such difficulty is attributable to several underlying causes. Species are described only from the male imago or if larvae are known, they may be difficult
or impossible to separate from congener. There remains a continuing need for rearing to associate immature stages with known adults, although collection of pharate material (pupa in larva, adult in pupa) can allow association without rearing, as can the use of molecular diagnostic techniques (see e.g. Ekrem et al. 2007; Stur & Ekrem 2011).

In a previous key (Wiederholm 1983) multiple authors sought to present to ecologists and others who needed to identify chironomids, but who were not necessarily specialists in the taxonomy of the family, keys and detailed generic diagnoses. Although based primarily on material from the Holarctic region, this made the work of value also to a new generation of taxonomists. For this new work, all diagnoses have been revised and include larval features of all described taxa with adequately known larvae in the Holarctic region. Genera included in this work are those clearly defined and accepted by most contemporary workers. Several have become better understood since the first edition of the key, and inevitably some new ones have been defined in the intervening period.

An important point to note is that in all cases, keys and diagnoses are based on fourth (final) instar larvae only. In general they will also work well for third instars but caution should be exercised, especially in relation to quoted ratios which vary considerably between instars. Similarly the size classes which are given relate to the body length of fourth instar larvae and are as follows; small < 5 mm; medium 5–10 mm; large 10–20 mm; very large >20 mm.

Here we treat the subfamilies in the following order: Buchonomyiinae, Podonominae, Tanypodinae, Telmatogotoninae, Diamesinae, Prodiamesinae, Orthocladiinae and Chironominae, following Cranston et al. (2011). Within Tanypodinae, the tribe Pentaneurini is recognised, with all other genera treated as ‘non-Pentaneurini’ pending review of their internal relationships (ongoing molecular studies suggest respective monophyly of the two groups). In Chironominae, tribe Tanytarsini is recognised, with the remainder treated as Chironomini due to doubts concerning monophyly of ‘tribe’ Pseudochironomini. Genera are dealt with in alphabetical order within each major group (subfamily or tribe as noted above). The subfamilies Aphroteniinae (Australia, South Africa, South America), Chileno-myiinae described by Brundin (1983) from South America, and Usambaromyiinae described by Andersen & Sæther (1994) from East Africa are absent from the Holarctic region: amongst these, larvae are known only for Aphroteniinae (Brundin 1966; Cranston & Edward 1992).

Collection of material

In ecological work the type of sampling technique employed depends upon a variety of considerations, notably the type of water body and nature of the substratum to be sampled and the objectives of the particular investigation. A great variety of sampling devices have been employed and it is neither appropriate nor practicable to review them here.

During the last two decades subfossil head capsules of chironomids have been widely used in reconstructing climatic changes during the last 10,000 years by taking core samples from bogs and lake beds. Methods used in collecting and processing such samples can be found in e.g. Walker (1987, 2002).

Preservation and mounting

Chironomids, whether larvae, pupae or imagines, are preserved most conveniently in 70–80 % ethanol or isopropanol and if stored in liquid, should be kept cold and dark. Material intended for molecular studies, especially larvae and pupae should be preserved in ethanol or isopropanol of higher concentration (96–100%) as they contain more water that will dilute the preservative. Formalin should not be used even for the shortest period; material preserved in this way is useless for slide-preparation, and, as with ‘denatured’ ethanol, DNA is degraded making it unavailable for molecular study. For longevity of material, slide preparation in a permanent mountant such as Canada Balsam or Euparal is preferable, especially for taxonomic studies or vouchers from ecological studies. Dried and pinned adult material has historical value but for the short-term wet preservation is preferable if only on the grounds of convenience. Larvae usually are killed by immersion in alcohol which causes the mouthparts to contract, especially in higher strength alcohols, which also make specimens brittle and prone to lose diagnostic setae. Better slides can be produced from larvae killed by placing them in weaker alcohol, or in clean water which is slowly heated to near boiling, or cooled in a refrigerator freezer, thereby relaxing the mouthparts.

Although it is certainly possible to make identifications on unmounted material, especially where the user is familiar with a modestly diverse regional fauna, microscopic examination usually is necessary. For detailed examination specimens need to be mounted with care on microscope
slides. Specimens intended for long-term storage in voucher collections or for which expert help is to be sought should preferably be mounted in either Canada Balsam or Euparal. In the case of whole larvae, the head capsule should be removed and macerated in a warm 10% solution of caustic potash (KOH) for 5 to 10 minutes. The specimen then is passed through glacial acetic acid (5 minutes), alcohol (e.g. ethanol or isopropanol) (15 minutes) and alcohol layered over either cedarwood or clove oil (15 minutes) before mounting in Canada Balsam. If Euparal is used as a mounting medium the alcohol/cedarwood oil stage can be omitted, with mounting of the cleared specimen directly from high strength alcohol (ethanol or isopropanol).

The head and body should be mounted under separate cover slips; otherwise the mount is likely to be too thick to permit examination of the head capsule using oil immersion. By applying gentle pressure and carefully moving the cover slip from side to side, whilst observing the results through a dissecting microscope, it is a relatively simple task to orientate the head so that it lies horizontally with its ventral side uppermost. In the Chironominae it is preferable to remove the dorsal surface of the head, together with the labrum in order to observe the dorsal sclerites. In the Tanypodinae the complete head should be cleared such that both ventral and dorsal setae (and pits) can be seen without being obscured by uncleared head contents. In the Orthocladiinae it is often necessary to have a well laid-out labrum-epipharynx to observe the structures in detail.

When dealing with reared series of an imago together with pupal and larval exuviae it is essential that all stages should be mounted on the same slide thus eliminating the possibility of future misassociation. Pupal exuviae often must have their abdomen and cephalothorax dissected before mounting in Canada Balsam or Euparal. Also the imago must be dissected and it is easiest if the parts are mounted under separate cover slips: five will usually suffice, the head and separated antennae being placed beneath one, the wings under a second and legs, abdomen (dorsal side uppermost for males, ventral side uppermost for females) and thorax (laterally) beneath three further cover slips. The abdomen requires maceration in hot 10% caustic potash (KOH) to render details of the hypopygium visible and together with the rest of the imago should be treated in the manner already described for the larva, before mounting in Canada Balsam or Euparal. Note that when working with molecular voucher materials, whole or partial body extraction methods should be used, without destruction of the specimen. Following extraction, a short soak in dilute KOH should be used to clear any remnant non-cuticular material, and the standard mounting procedure used for permanent preservation and subsequent curation.

When dealing with large numbers of larvae and if routine identification is all that is required, the process outlined above is unnecessarily complex and time consuming. Several mounting media are available which eliminate the need for dehydration and themselves serve as clearing agents and larvae may be mounted directly from weaker alcohol, or even from water. Optically they tend to be better than both Euparal and especially Canada Balsam. Several formulations are possible. Polyvinyl lactophenol, once widely used, is less acceptable on health and safety grounds. Berlese, Hoyer’s and related mountants are less dangerous to health and although easily made up from basic constituents, contain chloral hydrate which is increasingly difficult to obtain. The chief drawback of mountants other than Canada balsam and Euparal is their impermanence, with a tendency to crystallize on drying, which is a problem if re-examining slides after a period of several months or more. This can be overcome by ringing each cover slip with nail varnish or Euparal after the initial drying period. At ambient temperature Berlese mountants clear specimens, enhanced by drying in an oven or on a hot plate at 50°C. The optical qualities of Berlese is the best for photography, especially at highest magnifications, but all valuable specimens should be remounted in a permanent media (Canada Balsam, Euparal) for archival purposes.

**Slide collections**

A well organized, well curated slide collection is indispensable to validate records. Because the larvae of many species still are undescribed, slide mounted material can also be of great importance for further taxonomic studies. All (permanent) slides should thus be properly labelled with information about where the specimen was collected (country, region/state, municipality and place), the date when it was collected (i.e. 12.vii.2006) and the name of the collector (leg.). For the name of the place use a name which is found on a commonly used map or gazetteer. GPS or Google-map derived geographical coordinates should be added when known, as well as information about collecting method and habitat. Temporary codes should be just that — temporary — and should become detailed, promptly. Labels
can easily be printed on self-gluing stickers using a laser writer. When mounting the material only the right 2/3 of the slide should be used; the left 1/3 should be left for the label. Never place labels on the underside of the slide as it will increase the thickness of the slide and the stability when the slide is placed under a microscope.

If the head capsule and the body of the larvae are mounted under separate cover slips only one larva should be mounted on each slide to avoid later confusion about which head capsule belongs to which body. If more than one larva is mounted on the same slide, then the head capsule and the body should be kept underneath the same cover slip, but as mentioned above that might make identification more difficult. When reared material is mounted larva, pupa and the adult should be kept on the same slide, making at least seven cover slips necessary and the larva, pupa and the various body parts of the adult should be mounted in a strict order on the slide.

When drying the mountant tends to shrink and drops of fresh mountant should be added along the edge of the cover slips to fill up empty spaces if necessary. Try to avoid air bubbles. Particularly Canada balsam dries rather slowly at room temperature. To speed up the drying process the slides can be placed in an incubator at 50 to 60°C. However, when heated the mountant tends to become more viscous and the position of the body parts might change due to pressure from the cover slip, so slides should be checked and the position of the body parts adjusted if necessary before the slide is totally dry.

A slide collection can be organized in slide boxes with room for e.g. 100 slides. However, the slides need to be properly dried before stored in a slide box. The slide boxes should be stored vertically such that enclosed slides are held horizontally. If the slides themselves are stored vertically it is a risk that the various body parts end up along the lower edge of the slide.

**Identification**

The keys and diagnoses presented in these following chapters are sufficient for identification to generic or in some cases subgeneric or species group level. When there is a need to identify larvae to species it may be possible using specific regional keys to larvae such as those of Epler (2001) for south-eastern United States, Makarchenko & Makarchenko (1999) for Russia and sources mentioned throughout the following text. However it must be emphasised that it may not be possible to identify larvae to species without rearing at least to a diagnostic pupa, or male imago. Particularly for some pentaneurine Tanypodinae, a visible pupal thoracic horn within the swollen anterior segments of the late 4th instar larva is valuable indeed, perhaps even for correct generic allocation. Alternatively, larvae can be identified to species by comparison of their DNA with data in a reference library (i.e. DNA barcoding) or directly through genetic association with diagnostic life stages (Ekrem et al. 2007; Stur & Ekrem 2011). Sorting of individual larvae in the field may be desirable, unless the laboratory is close by. Many species, especially amongst the Orthocladiinae, are very susceptible to a rise in temperature above that of their normal environment. Furthermore, if transported together with quantities of sediment, larvae are liable to be damaged or killed by movement of particles, or by reduced oxygen levels through biological activity within the sediment. A vacuum flask, filled with clean water from their immediate environment, can be used to transport larvae, and Cranston (1979) found that under these conditions larvae could survive for several days. Alternatively larvae with a small amount of water in polythene bags tied so as to trap a quantity of air can be transported in an insulated box. Roback (1976) and Epler (2001) provided reviews of these methods. Mendes (2002) discusses rearing in more tropical situations with reference to particular genera.

**Association through rearing**

Although it is not necessary for the purpose of using these keys it is nevertheless often desirable to obtain reared series of imago with larval and pupal exuviae. In this case it is vital that there can be no possible doubt as to the association between an emerged adult and its subsequently collected larval and pupal exuviae. The best way of achieving this is by rearing singly, in individual containers. For this purpose well-developed fourth instar larvae, recognizable by their swollen thoracic segments, are preferable since a high level of success can be achieved in a relatively short time without the necessity of providing a food supply.

Many species, especially amongst the Chironominae and Tanypodinae will complete their development at normal room temperatures. Others, particularly many Orthocladiinae are very susceptible to raised temperatures and need to be
maintained in incubators at a temperature close to that of the environment from which they were collected. In general, providing the depth of water in the rearing vessel is not too great relative to its surface area, it is not necessary to provide additional aeration. Cotton-wool stoppered 50 x 10 mm glass vials are easy to handle and inspect when in large numbers. Containers must be examined daily, since adults, which are short-lived, rapidly decompose if they fall back onto the water surface after death. Newly-emerged adults often do not make good microscope slide mounts. A useful technique is to place the imago in a small vial with a piece of moist filter paper and to keep the vial, plugged with cotton wool, in a cool, dark place for 24–48 hours to allow the cuticle to harden.

Midges which fail to complete their development under these conditions most commonly die as a pupa. In this case it can be useful to dissect out at least the genitalia of the enclosed imago, or, if development has not proceeded sufficiently, identification may often be effected using pupa characters alone. As mentioned above, pharate material is valuable and should be retained.

Association and identification through genetic comparisons

As online databases that include genetic data on chironomids grow, identification of larvae through comparisons of commonly sequenced genes (e.g. Cytochrome c oxidase subunit 1, COI) will become an effective way to obtain species-level identifications. Obtaining partial COI-sequences from chironomids is usually straight-forward through standard PCR with the Folmer primers (Folmer et al. 1994) as long as the specimens have been properly preserved (Krosch & Cranston 2012). It is crucial, however, that databases intended for species recognition are well curated and that the data they contain are based on expert identifications of vouchered material. One such database is the Barcode of Life Data Systems (www.boldsystems.org; Ratnasingham & Hebert 2007) that currently holds DNA barcodes from more than 18,000 specimens of more than 1000 Chironomidae species (accessed January 2013).

Several recent studies have demonstrated the effectiveness of using DNA barcodes to identify species and associate life stages in Chironomidae (Ekrem et al. 2010; Stur & Ekrem 2011, and references therein), and there is little doubt that this method will contribute significantly to the knowledge of chironomid immatures in the future. As DNA barcoding of bulk environmental samples becomes more common (Hajibabaei et al. 2011, 2012), it is also likely that chironomid larvae will be more frequently included in aquatic monitoring and freshwater assessments.

Illustrations

In this edition we follow the precedents of the previous one, and the overwhelming majority of taxonomic publications, in using ink / line drawings. These have the advantage of allowing subtle emphasis of features of significance, and downplay of those that are less important in identification. However, the downside can be that inexpert users have difficulty in interpreting line drawings when faced with ‘real specimens’ and thus there have long been moves towards complementation or even replacement by photographs. The wide availability of digital image cameras and ‘stacking’ of images to produce deep focus images has seen a dramatic improvement in image quality. It is commonplace to see descriptive and identificatory studies illustrated with coloured, in depth, images of structures, especially those available via the internet (e.g. Cranston 2010). Nevertheless, even with ability to view (and image) hyaline structures with Nomarski interference optics, line illustrations will continue to have an important role in interpretation of ‘3D’ morphological structures.

References


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