

# A digital reference collection for aquatic macroinvertebrates of North America

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**Abstract:** Aquatic invertebrates are a key component of freshwater ecosystems, and understanding aquatic invertebrate taxonomy is a cornerstone of freshwater science. Physical reference collections of expertly identified voucher specimens are the ‘gold-standard’ used to confirm specimen identifications. However, most biologists lack access to such collections, which themselves tend to be highly regionalized and somewhat limited in terms of taxonomic scope. The North American Aquatic Macroinvertebrate Digital Reference Collection (NAAMDRC; <https://sciencebase.usgs.gov/naamdrc>) was developed by the US Geological Survey (USGS) to overcome these limitations of physical collections. NAAMDRC provides users with public-domain, high-quality digital photographs to help verify specimen identifications.

**Key words:** digital microscopy, benthic invertebrates, museum collection, voucher collection

Physical reference collections (a collection of specimens that have been identified by taxonomic experts) are important tools for confirming specimen identifications, and calls by taxonomists for digitizing these collections are increasing (Ang et al. 2013). Digital reference collections (DRCs) complement physical collections in several ways. DRCs are available to virtually everyone via the worldwide web, whereas physical collections are typically accessible to only a small number of researchers. DRCs can cover greater taxonomic diversity compared to physical reference collections, which tend to be regional and somewhat limited in scope (Ang et al. 2013). Furthermore, physical specimens have intrinsic value associated with them in terms of their utility, yet they are inherently fragile. Digitizing these specimens protects them from possible damage or loss incurred by repeated handling.

Here, we introduce the North American Aquatic Macroinvertebrate Digital Reference Collection (NAAMDRC; <https://www.sciencebase.gov/naamdrc>) as a tool to help verify the identification of aquatic macroinvertebrate specimens. An understanding of aquatic invertebrate taxonomy is required for many topics within freshwater science (e.g., community and foodweb dynamics and trait-based studies) and for applications such as bioassessment. Other DRCs for aquatic macroinvertebrates have been published online, but they tend to be regionally based (e.g., California Department of Fish

and Wildlife 2017), focused on a particular taxonomic group, such as Chironomidae (e.g., Cranston 2010), or somewhat limited in taxonomic scope (Carnegie Museum of Natural History 2017). Most specimens in NAAMDRC (pronounced nam-dirk) are presented at genus level because immature stages are most often encountered in freshwater science, and species-level keys are unavailable for immature stages of most aquatic insects. NAAMDRC serves as a clearinghouse of high-quality, public-domain images for use in scientific presentations and publications by the broader freshwater science community. Our goal is that this collection will be used as a tool by researchers and practitioners of freshwater science to: 1) advance freshwater research that depends on accurate identification of aquatic invertebrates, 2) enhance teaching of aquatic ecology in kindergarten–high-school (K–12) and university classes, 3) aid training of graduate students conducting freshwater research, and 4) support resource management agencies and private-sector companies in processing aquatic macroinvertebrate samples.

## CONSTRUCTION OF NAAMDRC

We obtained macroinvertebrate voucher specimens for the DRC through 3 primary sources: our own reference collection at the US Geological Survey (USGS) Aquatic Exper-

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imental Laboratory (Fort Collins, Colorado, USA), the USGS National Water Quality Laboratory (Denver, Colorado), and the C. P. Gillette Museum of Arthropod Diversity at Colorado State University (Fort Collins). This 1<sup>st</sup> rendition of NAAMDRC includes taxa primarily in the western USA (i.e., those occurring west of the Mississippi River). However, because genera are generally widespread, the collection includes many taxa that occur in the eastern USA, Canada, Mexico, and elsewhere (e.g., Holarctic taxa). The current emphasis is on the aquatic insect orders: Coleoptera, Diptera, Ephemeroptera, Hemiptera, Lepidoptera, Megaloptera, Neuroptera, Odonata, Plecoptera, and Trichoptera (Fig. 1A–I). Most digitized specimens represent immature life stages, but in some cases, images also are available for pupa and adult stages. An annotated list of the collection holdings, including life stages, is maintained on the NAAMDRC website and is continuously updated as taxa are added.

We photographed specimens with 3 models of INFINITY™ series cameras (models 1-2C, 3-3URFC, and 2-1RC; Lumenera Corporation, Ottawa, Ontario) coupled with either stereomicroscopes (model EMZ-TR; Meiji Techno, San Jose, California; model M8, Wild Company, Heerbrugg, Switzerland) or a compound microscope (model Vanox; Olympus, Waltham, Massachusetts). We photographed specimens >2 mm in length superimposed over a 1-mm gridded stage to provide spatial reference when comparing other individuals for verification (Fig. 1A–I). We photographed taxa from multiple views (e.g., dorsal, ventral, and lateral; Fig. 2), and we annotated  $\geq 1$  photograph in each series to illustrate important family- or genus-level characteristics noted in commonly available dichotomous keys (e.g., Merritt et al. 2008). We photographed slide-mounted specimens from the family Chironomidae with a compound microscope zoomed between 100 and 400 $\times$  magnification.



Figure 1. Insect orders represented in the North American Aquatic Macroinvertebrate Digital Reference Collection (NAAMDRC). A.—Coleoptera. B.—Diptera. C.—Ephemeroptera. D.—Hemiptera. E.—Lepidoptera. F.—Megaloptera. G.—Odonata. H.—Plecoptera. I.—Trichoptera.



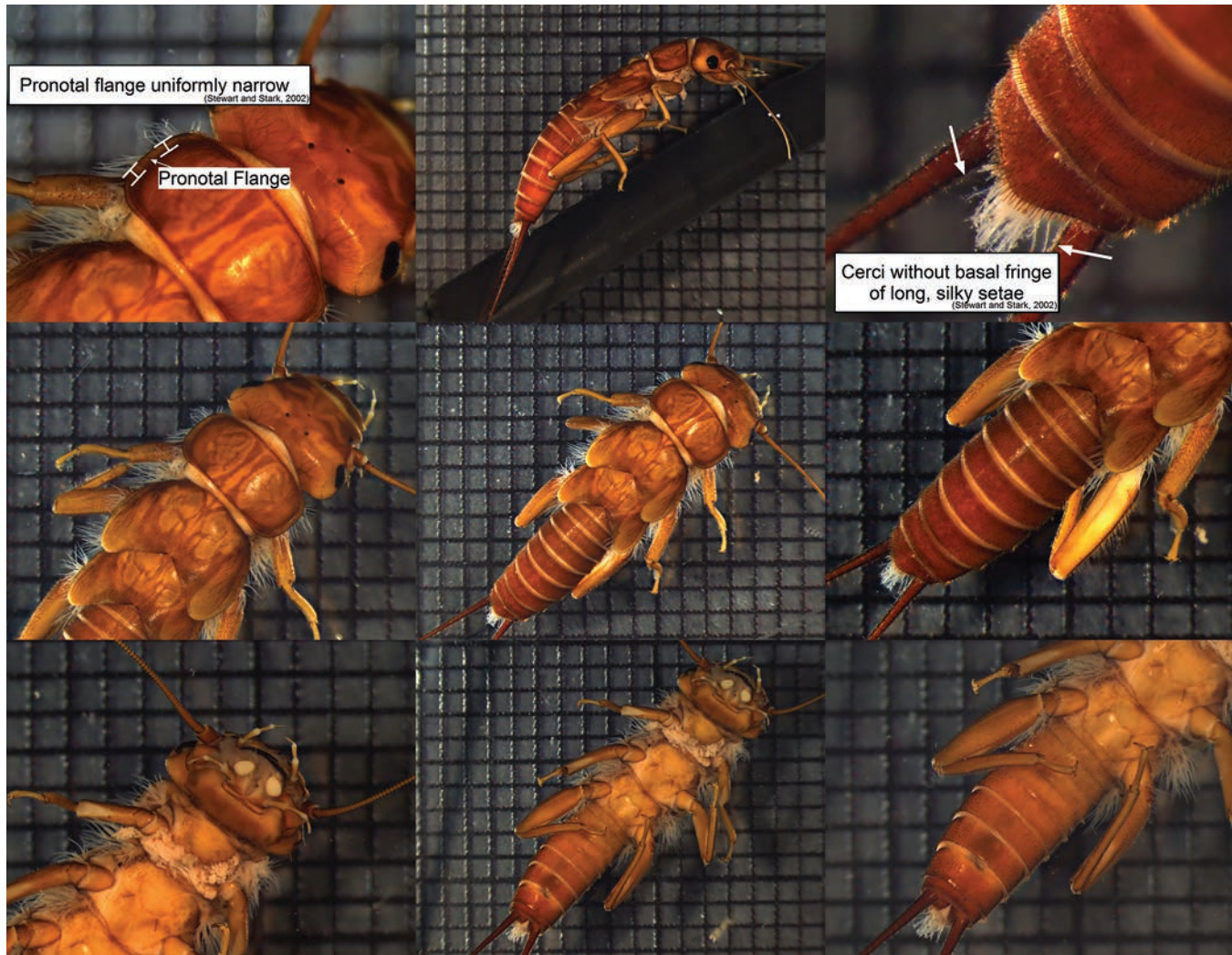


Figure 2. Standard range of plates for a single taxon, the plecopteran genus *Beloneuria*, illustrating the different aspects available and including 2 plates annotated with the final identifying characteristic from the terminal couplet in a dichotomous key.

These images emphasize ventral aspects of the head and associated structures (e.g., mentum and antenna) that are important for chironomid identification (Fig. 3A–E).

The shallow depth of field associated with digital microscopy prevents all surfaces and structures from being in focus within a single image. We used ‘focus-stacking’ software Zerene Stacker (version 1.0; Zerene Systems, Richland, Washington) and INFINITY ANALYZE™ (version 6.5.4; Lumenera Corporation, Ottawa, Ontario) to merge multiple images taken at variable focal lengths to produce a single, in-focus image. Specimens or structures that were too large to capture in a single field of view were combined into a composite image using the open-source GNU Image Manipulation Program (GIMP; version 2.8.22; The GIMP Team).

#### NAVIGATING AND USING NAAMDRC

Users can view the collection by clicking on the ‘specimen list’ for tabular format or the ‘collection’ button for

graphical format. The collection is organized hierarchically, wherein taxa are displayed phylogenetically by order and then alphabetically by family and genus. Taxa included in the specimen list are directly linked to images. The user can enter the collection by clicking the ‘collection’ button or by clicking within the search bar. This action displays taxon cards for the entire collection that are organized hierarchically as described above. The taxon cards include a thumbnail photograph and a citation for any dichotomous keys that were used to annotate images for that taxon. The user can scroll through a thumbnail gallery of the taxon within the card by clicking on the margin of the photograph. This same set of images can be viewed in full-screen mode by selecting the ‘slideshow’ button on the taxon card. The user can download images within the slideshow directly to their device by using the ‘download image’ button.

The user can query the collection by using the search box or the taxonomy filters. The user can click within the



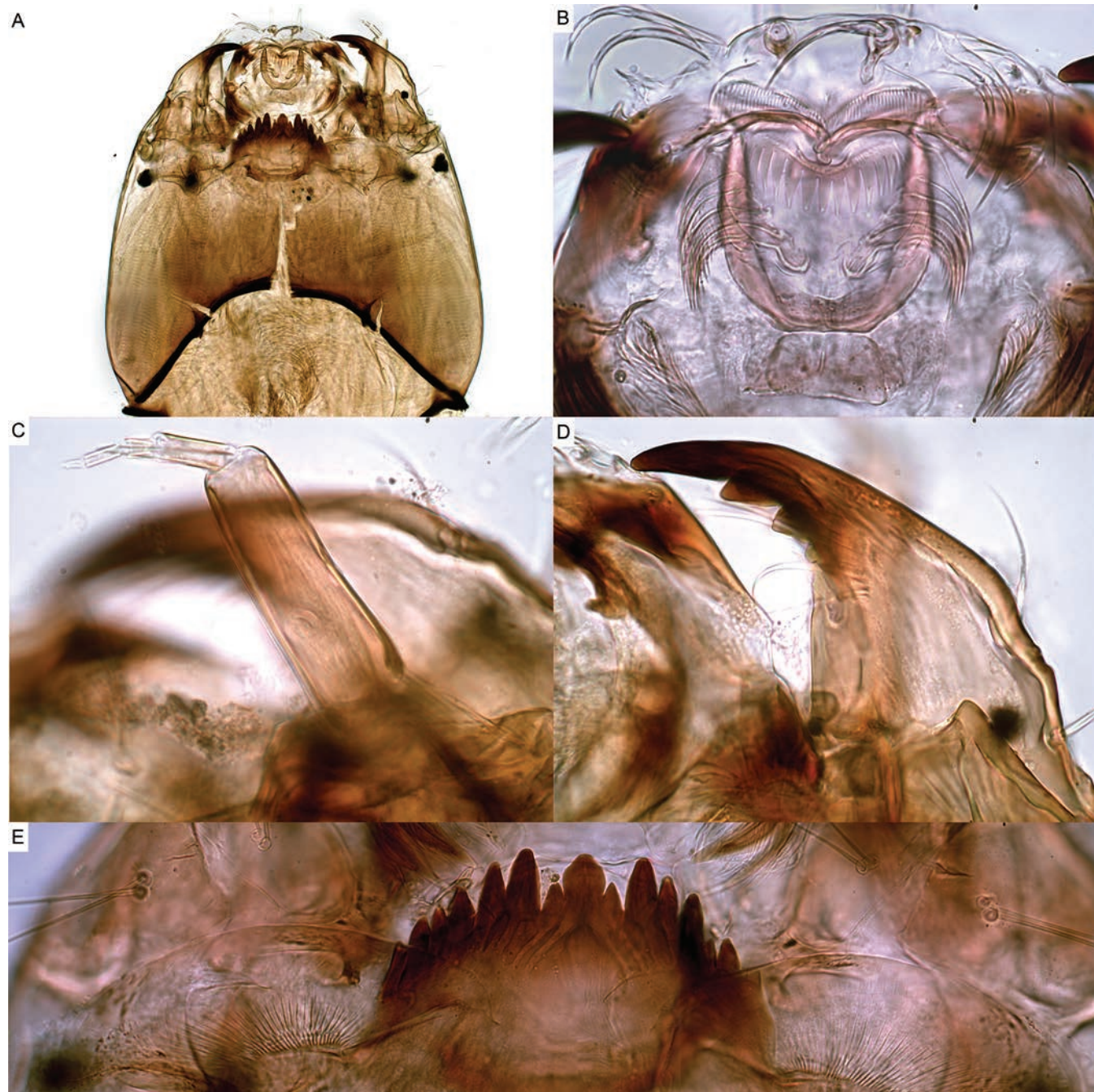


Figure 3. Typical set of images illustrating diagnostic characteristics of chironomids shown for the genus *Chironomus*. A.—Head. B.—Epipharyngeal region. C.—Antenna. D.—Mandible. E.—Mentum.

search box to view an alphabetized drop-down menu for the entire collection and then type within the bar to sort the drop-down menu or manually scroll through the list to make a selection. The user also can query the collection by using combinations of order, family, and genus. For example, if the user selects the order Coleoptera from the ‘filter by order’ search box, then only taxon cards associated with that order will be displayed, and only families within that order

will be included under the ‘filter by family’ drop-down menu. The user can further filter the collection by life stage by toggling the ‘larva’, ‘pupa’, and ‘adult’ buttons on the tool bar.

NAAMDRC is intended to assist users to identify or verify specimen identifications while concurrently using dichotomous keys and other diagnostic literature. We present 3 hypothetical cases below illustrating how NAAMDRC images can be used by the scientific community.

- 1) A research group studying the effects of contaminants on stream invertebrates just completed enumerating and identifying specimens collected from a region beyond where they normally work. As part of their internal quality-assurance policies, a voucher collection of unique taxa assembled from project samples are verified by a 2<sup>nd</sup> internal taxonomist and by a Society for Freshwater Science (SFS) regionally certified taxonomist from an independent laboratory. The project was conducted in an unfamiliar region, so the researchers encountered several genera that were 'new' to the research group. Therefore, in addition to using available dichotomous keys and supporting literature to make determinations, the 2<sup>nd</sup> internal taxonomist compared their specimens with NAAMDRC images before sending the voucher collection to the independent laboratory.
- 2) An undergraduate university student enrolled in an aquatic entomology course uses NAAMDRC images to help study for weekly laboratory quizzes and to verify specimen identifications within their personal collection compiled over the course of the semester.
- 3) A graduate student is putting together a presentation for the next SFS meeting and wants to use images to highlight important taxa from his or her research. The digital images housed by NAAMDRC are public domain, so this student can simply search for the taxa of interest, download selected images, and insert these images into the presentation.

#### FUTURE DIRECTIONS FOR NAAMDRC

The next step is to expand the geographic and taxonomic scope of NAAMDRC to include all aquatic invertebrate taxa (including other noninsect groups) in North America. We will partially rely on contributions of specimens from other researchers for this effort, and instructions for specimen contribution are included in the NAAMDRC website. Other possibilities include linking distribution data (e.g., MAPIT 2017, USGS 2017) and ecological-trait information (e.g., Vieira et al. 2006) to each taxon so that this collection can serve as a 'digital ecological atlas' of aquatic macroinvertebrates of North America.

#### CITING NAAMDRC

The photographs in the NAAMDRC are public domain and are available for use without permission. If these images are used in public presentations, such as scientific meetings, please provide photo credit (e.g., USGS NAAMDRC) for images. If images are used for publication or if NAAMDRC was used to support published research (e.g., as a specimen identification tool to augment traditional taxonomic keys), then please cite this journal article.

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