Sampling and counting procedure for subfossil chironomids (Diptera::Chironomidae) from lake sediment

Marcus Thomson
University of California, Santa Barbara
National Center for Ecological Analysis and Synthesis (NCEAS)
zizroc@nceas.ucsb.edu
28 February 2020

This document is meant as an aid to chironomid processing for paleolimnological analysis. It is based on the procedure I was taught by Prof. David Porinchu, University of Georgia, Athens, with small tweaks based on my own experience based on my PhD dissertation work, analyzing subfossil chironomids from late Holocene lake sediments from Utah.

Contents

Materials

- 2× stereo microscopes (“stereoscopes”), with 10-40× magnification
- High-quality laboratory optical microscope with camera
- KOH solution (8% M)
- 250 mL beaker
- Hot plate
- Sieve
    - The sieve was built from a 10x10 cm piece of monofilament screen mesh (with 95 micron aperture size) and a small Nalgene bottle. Cut the top ⅓ from the top of the bottle, and hollow-out the center of the cap, taking care to maintain smooth edges with a sharp blade (e.g., box-cutter). The piece of screen mesh was

placed across the open bottle-top, and held firmly in place by screwing down the hollowed bottle-top. Take care to minimize stretching of the screen mesh.

- Modified syringe
    - Cut the narrow injection-tip off of a 1 cm diameter syringe.
- Erlenmyer flask with dropper containing distilled water
- Bogorov channel tray
- Counter
    - Use any hand-held analog counter. Also possible to use a smartphone app.
- Glass microscope slides
- Round slide cover slip
    - Square cover slips will work but round cover slips seem to be easier to manipulate when it comes to mounting the picked head capsules.
- Entellan® mounting agent
- High-precision microscopy tweezers
- Wooden stirring dowels
- Parafilm®
- Dichotomous key
    - There are many available in publications and online. I also found the site Chiro Key (http://chirokey.skullisland.info/) invaluable. Finding an appropriate key is a valuable investment in time.

Chironomid procedure

Preparation

You will need: 250 mL beaker, hot plate, sieve, modified syringe, distilled water, and KOH solution. You may also want a glass stirring rod and dissection spatula handy.

1. Use the modified syringe to subsample 0.5 mL of sediment. Place the sediment in a labelled 250 mL beaker containing ~100 mL of 8% KOH solution.

   NB: The volume of sediment necessary for your analysis will depend on the local concentration of subfossil chironomids. A good rule of thumb, which is ultimately time-saving, is to start by subsampling 1.0 mL of sediment, and reduce to 0.5 mL if you find the concentration to be sufficiently high. The greater the number of chironomids, the greater the significance of your findings; but for practical purposes, aim for at least 60 head capsules in sum.

2. Deflocculate the sediment in the KOH solution at ~70ºC for 30 minutes.

   NB: You may also stir gently to break up clumps. This is especially common for the top of a core and with gyttja. Take care not to crush sediment against the beaker walls, as

this may damage or disarticulate specimens.

3. After the deflocculated sediment has cooled, pour it through the sieve. Discard the filtrate, and keep the material that has accumulated at the base of the sieve. Use distilled water liberally to thoroughly rinse material from the beaker through the sieve. Rinse until the beaker is clean.

   NB: If the sediment has maintained some clumps, try and use the water jet to break them up. You may also need to use the tip of a glass stirring rod to encourage sub-95 micron particles through the sieve. The chironomid head capsules of interest (i.e., 4th instar stage) are significantly larger than the scale of the filter mesh. You will not lose specimens (whole or fragmentary) if you are gentle.

4. Place the clean beaker on the lab bench and invert the sieve over it. Use a few strong jets of distilled water to rinse the filtered material back into the beaker.

   NB: You should try and minimize the volume of water you add back into the beaker, because doing so will save you time later. However, for good results, it is critically important that you wash all of the sieve's contents into the beaker. Optimizing this step takes practice. Spend several seconds making a close inspection of the sieve, which should now be clean of macroscopic particles.

   Now your subsample is prepared for the "picking" stage. It is also stable enough to be stored for a few days in a cold room if necessary. Confirm that the beaker is properly labelled and cover the sample with Parafilm®.

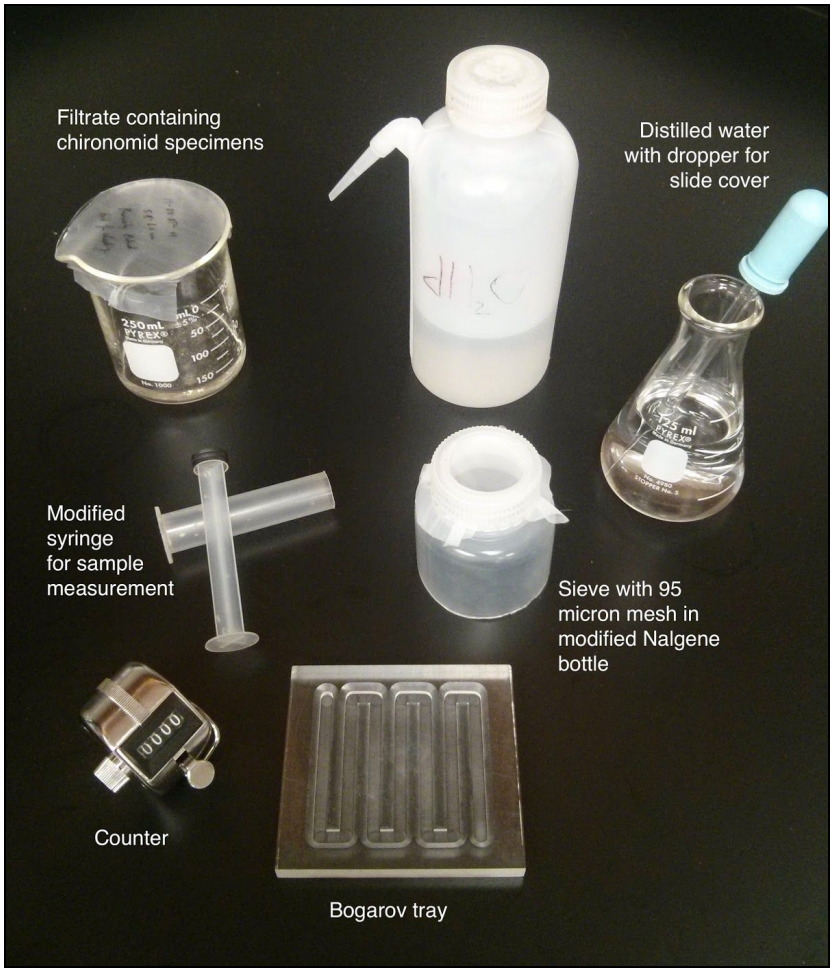

Figure 1. Equipment used for subsample preparation and chironomid picking.

Chironomid picking

In addition to the solution prepared above, you will need: Bogorov tray, counter, 2 dissection microscopes, microscope slides and slide cover slips, Erlenmyer flask and dropper with distilled water, tweezers, and chironomid picking record sheets.

You should place the dissection stereoscopes side by side on your lab bench with your chair between them. Take some time to find a comfortable position for yourself with the stereoscopes, as you will be shuttling between them. For the purpose of this procedure, I will label the left-hand stereoscope #1, and will use it for “picking”; the right-hand stereoscope #2, and will use it for “placing”. You will use #1 to find and pick out chironomid head capsules from solution in the Bogorov tray using the tweezers, and use #2 to place the specimens on a slide cover slip that you have prepared.

This is the most tedious part of the process. Although it will feel less so with time and practice, you should not forget to build breaks into your workflow. Beyond the eye-strain caused by too much microscopy, your selection skill will suffer over time, potentially introducing a selection

bias in the specimens that tend to be in the top trays to those that tend to come in the bottom trays. A good idea is to set a timer and break every hour or so, as well as periodically stop to focus your eyes on some object across the room.

1. First place a labelled microscope slide on the stage of stereoscope #2. Place a coverslip on the slide, such that it is centered in the stereoscope's field of view. Use the dropper to add a single drop of water to the center of the coverslip. The droplet should be large enough to cover ⅔ of the surface area of the coverslip, but should not be in any danger of over-spilling the edge and onto the slide.

2. Stir the beaker such that macroscopic particles are well mixed and then pour some of the solution into the Bogorov tray. The solution should fill the tray's channel to about half-height. Place the tray on the stage of stereoscope #1.

   NB: The Bogorov tray's shape utilizes the surface tension of water to inhibit the mixing of particles, so that you can proceed systematically along the channel and extract specimens of interest. This involves a good deal of patience and practice, as there will be many irrelevant specimens -- even other fossil insects -- present with the chironomids. Until you can comfortably ID whether a specimen is or is not a chironomid, pick everything you think might be. The height to which you fill the tray can help. Specimens will separate roughly on the floor of the channel and at the surface, usually along the edges. Filling to half-height means that you will usually need to use only 2 different field-focus settings, and chironomids are less likely to spill out of the channel.

3. At #1, start systematically at one end of the tray's channel (e.g., top left). Set the field-focus to the top surface of the solution in the channel and begin to scan. To scan, the tray should be at rest and any water waves should have dissipated; use your eyes only to quickly find several chironomids.

4. Bring the points of your tweezers slowly into your field of view, and pick out one specimen at a time. Use the counter to record the number of specimens you have picked: 2 clicks for an entire head capsule, and 1 click for any fragment thereof.

5. Turn to #2, and place the specimen into the water droplet on the coverslip. You should see the specimen pop up onto the surface of the droplet, and likely slide down to its edge under gravity. A coverslip can accommodate about 2 dozen specimens.

   NB: Many people find the most difficult part of this step is getting the specimen to detach from the tweezers and deposit in the water droplet. This is almost always because they are squeezing too hard when picking. Experiment with techniques to inhibit yourself from doing this. With practice, you should be able to pick and place specimens with ease. If stuck, you can use a second pair of tweezers to scrape the specimen off; but after doing so, you should wipe down your tweezers with a Kim-Wipe to remove any residue.

The next most difficult part is keeping the specimens separated on the coverslip. As the droplet evaporates, specimens will naturally deposit in concentric rings. But they will also tend to electrostatically attract one another; so if you place them close together, they will clump. This can make identifying taxa more difficult later. A good technique is to place subsequently picked specimens on opposite sides, or separate quarters, of the droplet.

Each microscope slide can accommodate 2-4 coverslips, depending on size. When a slide is complete with coverslips full, confirm its labelling, number it, and set it aside to dry. In addition to labelling your slides with the correct subsample depth, it is critical to number it. When you complete a subsection, record the total number of slides on each.

6. Turn to #1, repeat the previous 2 steps until the field of view is cleared of relevant specimens. When complete, refocus the stereoscope to the channel floor, and pick all relevant specimens from this level, placing them on the coverslip on #2.

7. After a section of the channel is clean, advance by moving the tray, and refocusing on the surface such that a new section is clearly seen in the field of view. Repeat the previous steps, picking from #1 and placing on #2 until the new section is clean, surface and floor.

8. After you have picked all of the specimens from the Bogorov tray channel, do a scan of the top surface of the tray. Occasionally specimens appear here, perhaps due to overflow of the channel or because they were dropped before being placed. After a tray is complete, empty the channel and, as before, add solution from the beaker.

9. Completing a subsample means finishing all of the solution in the beaker, rinsing with a little distilled water, and doing a final tray. Depending on how much water you used to rinse the sieve, this could be several trays.

Mounting

In addition to the microscope slides prepared above, you will need: gloves, Entellan, wooden dowels, paper towel, and tweezers. (High precision tweezers are often damaged in the course of chironomid picking. The mounting step is a good use of tweezers whose tips are too damaged for picking.)

Allow your coverslips to dry completely before attempting to mount on the slides, and use only fresh mounting agent. Mounting wet slides or using expired mounting agent can lead to difficulties identifying taxa. If any chironomids fell from a coverslip to a slide, use the stereoscope to replace them. Finally, give yourself lots of space on the lab bench, use gloves, and set yourself up in a well ventilated area. Entellan contains xylene, which you should read about on the MSDS prior to handling.

1. Set each slide about 10 cm apart on the bench, on pieces of paper towel to catch mistakes. Use tweezers to carefully push each coverslip to a near edge of the slide, such that it overhangs the enge by about ⅓ of its diameter. See fig. 2A.

2. Use a clean wooden dowel to drip several mL of Entellan onto a folded paper towel on the bench. This will be the reservoir from which you will add the mounting agents to the coverslips. Close the Entellan bottle again to avoid its contamination.

3. Use the wooden dowel to add a few drops of Entellan from the reservoir onto each of the coverslips. Your slide should look like fig. 2B.

4. Using your tweezers to grab the dry, overhanging edge of each coverslip, and flip it along across the long axis of the slide such that you place it down onto the slide with the mounting agent and chironomids sandwiched between the coverslip and underlying slide. Tap the coverslip very lightly with the tweezers to level it. Your mounted slide should look like fig. 2C.

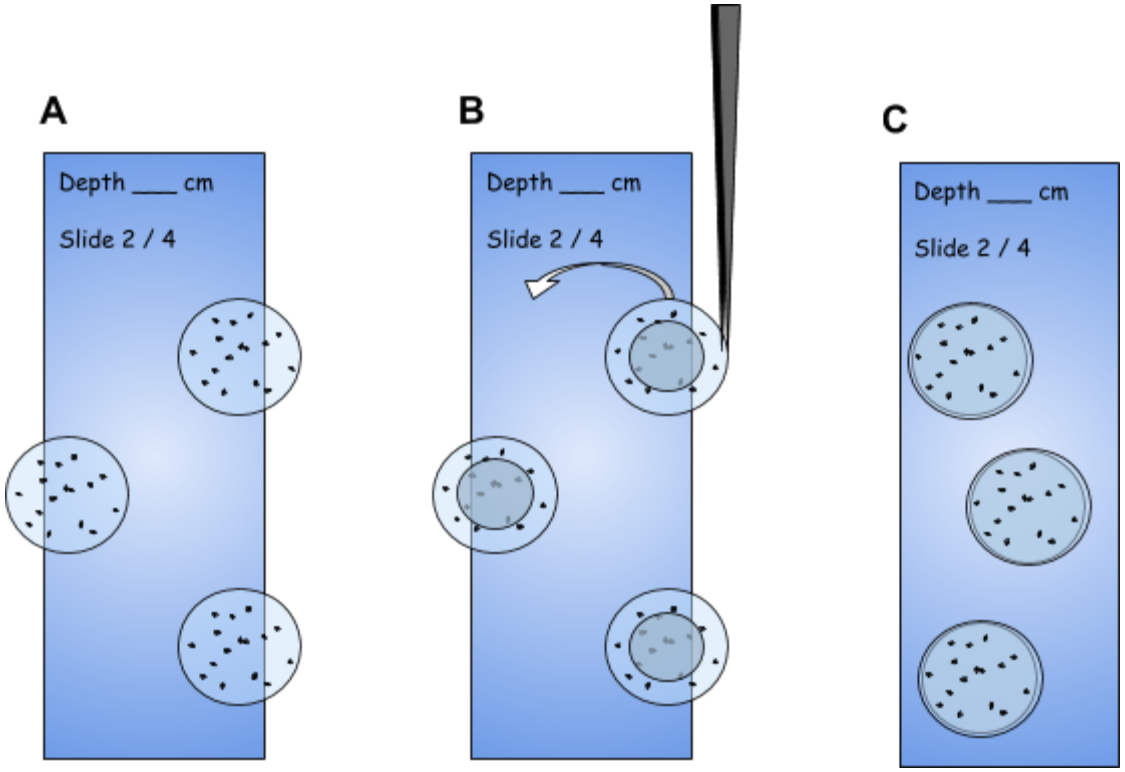

Figure 2. Steps in the mounting procedure for chironomids on circular coverslips onto an arbitrarily labelled slide. (A) Three dry coverslips with chironomid specimens placed along the edges of a microscope slide; (B) droplets of Entellan added to the center of each coverslip, with tweezers positioned to flip the coverslip; (C) coverslips having been flipped and mounted on the slide, with the correct amount of Entellan having been used.

5. Put your mounted slides away in a dry, protected space such as under a fume hood and allow them to set for 24-48 hours.

Troubleshooting: It is a good idea to practice on some dummy slides first. Even for practiced hands, 3 common problems to arise when mounting the coverslips are (1) trapping air bubbles under the coverslip, (2) making a mess of the Entellan, and (3) cracking the coverslip. I find that the method described above is more successful at making clean, bubble-free mounts than adding the droplet of Entellan to the slide and flipping the coverslip onto it; however, it is a matter of personal comfort. If you do make a mess of the mounting, and some mounting agent and/or specimen is pushed out from under the coverslip, do not try and fix it. Simply let it set. Making the taxonomic ID will be more difficult through the optical distortions caused, but not impossible. If the slide cracks in the middle of the mounting process, attempt to place the pieces to be as flat as possible. This will reduce optical distortions and help push any air bubbles to the edge.

Taxonomic identification

In addition to the mounted slides, you will need: optical microscope with quality camera, taxonomic keys, chironomid counting sheets.

1. Pick several subsamples at widely spaced depths from your sediment core. If you have other information about major transitions in the core (e.g., indicated from LOI, magnetic susceptibility, etc.), pick subsamples that cross these. To begin, you are aiming to become familiar with the appearance and variety of subfossil assemblages that occur through the core.

   NB: Your goal is to minimize biases. You will almost certainly repeat the taxonomic identification of these early subsamples, which will go some way to eliminating the bias caused as your ID skillset improves over time.

2. Use a new tally sheet for each slide, not for each subsample. Later, you will attach all of the filled tally sheets together for each subsample. Fill in the top of the tally sheet, with careful attention to subsample ID and slide number. Make a quick sketch of each slide at the top of the tally sheet, which you will use to keep track of which coverslips you have completed, and which remain.

3. Proceed in a systematic way (e.g., starting at top-left, at the labelled end of each slide) to find and ID specimens under each coverslip, as in fig. 3.

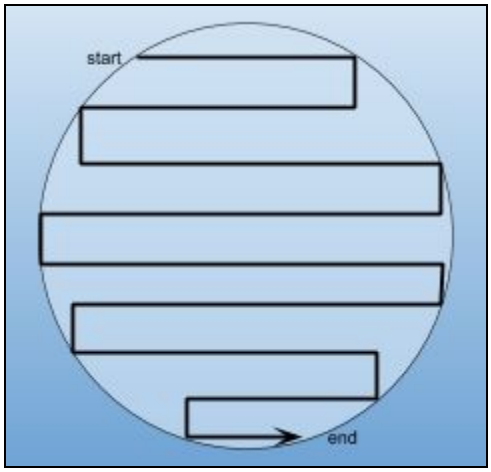

Figure 3. Boustrophedonic scanning direction for each slide-mounted coverslip.

4. To ID a chironomid, use a dichotomous key.

   NB: Chironomidae are taxonomically classified based on physical attributes. For fossil chironomids, which may have suffered diagenic changes, damage during subsample processing (which will appear as surface pitting), and mechanical disintegration, diagnostic attributes are usually scleric features on their head capsules, such as the mentum, mandibular “teeth”, antennae pedestal, and ventromental plate.

   Different sites can have slight differences in what will be taxonomically equivalent specimens from other sites. Use the blank lines in the chironomid tally sheet to add variant typologies until you decide later, based on a sufficient sampling of the assemblage, to merge taxa or not.

5. Some specimens may not be readily IDed at first. You will need to image these using the optical microscope’s camera. You should include a scale/scale bar and indicate the optical magnification. If it is the case that separate diagnostic features may not be resolved with the same focal depth, it is possible to generate an aggregated “flat” image from a layered set of images with different focal settings. The flattened image can be a helpful diagnostic tool.

Figure 4. Example of Dicrotendipes nervosus type. Identified from mentum-tooth structure and anteriorly crenate margins of ventrimental plates. Author's image.

Resources

Cranston, P.S. 2010. Chiro Key. http://chirokey.skullisland.info/

Cranston, P.S. 2010. Lucid key to Larval Chironomidae
https://keys.lucidcentral.org/keys/v3/Chironomidae/index.html

Walker I.R. 2001. Midges: Chironomidae and Related Diptera. In: Smol J.P., Birks H.J.B., Last W.M. (eds) Tracking Environmental Change Using Lake Sediments. Developments in Paleoenvironmental Research, vol 4. Springer, Dordrecht

Walker, I. R. 2007. The WWW Field Guide to Fossil Midges. http://www.paleolab.ca/wwwguide/

Chironomid picking record sheet

Name of preparer and date picked: _____ | _____ _____ | _____ _____ | _____ _____ | _____ _____ | _____		**Lake name** **Lake and drive code**
Sample depth min.-max. (cm) as given on Whirl-pak:	**Real sample depth (if different from left, cm):**	**Sample volume (mL)**

Date	**Counter number**	**Comments**

Notes and comments

Chironomid tally sheet

Researcher			Date			Sketch slide		
Sample depth			Slide #					
subfamily	count	∑	Chironominae	count	∑	Tanypodinae	count	
Orthocladiinae			Chironomus			Procladius		
Clunio			Cladopelma					
Corynoneura/ Thienemanniella			Constempellina					
Cricotopus/ Orthocladius			Corynocera ambigua					
Doithrix/ Pseudo-orthocladius			Corynocera oliveri					
Eukiefferiella/ Tvetenia			Cryptochironomus			Other	count	
			Dicrotendipes					
			Endochironomus					
			Microspectra					
			Tanytarsus					
						Notes		
			Chironom. subtotal					
Orthoclad. subtotal			Chironomid total					

This table should be completed with site-appropriate taxa.