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NOTE

The use of cotton blue stain to improve the efficiency of picking and identifying chironomid head capsules

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Abstract Cotton blue was added to sediment samples at least 2 h before chironomid head capsules were picked under a binocular microscope and mounted on slides for identification. The use of stain greatly increased the visibility of chironomid head capsules during picking and enhanced the contrast of various parts of the head capsules (pores, ventromental plates, striations on ventromental plates), which could aid identification. In the seven samples studied, there was no significant difference between the percentages of taxa found in stained and unstained samples. The number of taxa were also similar in stained and unstained samples. This method allowed samples to be picked faster.

Keywords Chironomid analysis · Staining · Improved identification

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Introduction

Chironomid (Diptera: Chironomidae) head capsules preserved in lake sediments can be used qualitatively and quantitatively to reconstruct environmental changes, including mean July temperature, oxygen, total phosphorous, salinity (Brooks 2006; Walker and Cwynar 2006) and macrophytes (Langdon et al. 2010). One factor limiting their wider use in high-resolution paleolimnological studies is the time involved in sample preparation. With the "standard" method (Walker 2001), many hours might be needed to prepare one sample, depending on the density of head capsules in the sediment, the number of head capsules required per sample, and the concentration of other particles larger than 100 μm in the sediment. Recently, various methods have been tested to decrease sample preparation time. Velle and Larocque (2008) added markers to calculate concentration and Rolland and Larocque (2007) developed a flotation technique. Verschuren and Eggermont (2007) successfully used a larger mesh sieve for samples from African lakes, but this technique was shown to be counterproductive for cold lakes (Larocque et al. 2010). Although these techniques decreased the time needed for sample processing, they share one critical time-limiting step–samples are sorted in a tray or dish under a stereomicroscope and each head capsule must be hand-picked for mounting on a microscope slide. The head capsules are often pale and may be mixed with remains of other organisms and sediment

particles that, depending on concentration, obscure head capsules and increase the picking time. This problem could be solved if chironomid head capsules could be stained uniquely, making them highly visible during the picking process.

Cotton blue has been used previously to stain chitin in fungal cell walls (Leck 1999). The stain was tested in this study for use with chironomid head capsules. To be considered successful, the method should (a) stain all chironomid head capsules, regardless of species, and (b) not compromise head capsule identification (i.e. all parts usually used for taxonomy should still be easily resolved).

Methods

Sediment samples from different lakes and different periods of time were used to test cotton blue as a staining agent for chironomid head capsules (Table 1). The standard method (Walker 2001) of preparation was used. 10% KOH was added to the samples overnight and samples were then sieved in a 100-μm mesh. Cotton blue stain was prepared by mixing 150 mg of cotton blue in 90 ml of 90% lactophenol. The amount of stain added to the sample varied with the volume of sample. Cotton blue was added until the sediment appeared blue. The stain was left in the samples for at least 2 h. Then, the residue was poured into a Bogorov tray and each head capsule was hand-picked under a Leica zoom 2000 stereomicroscope at 40× magnification. The head capsules were mounted in a drop of Hydromatrix on a microscope slide and identified with a Motic B3 professional compound microscope at 200–400×. Identification followed mainly Brooks et al. (2007) and Larocque and Rolland (2006). The Motic Images 2.0 Plus program was used to capture the images obtained with the Moticam 2000 camera mounted either on the stereomicroscope, when picking, or on the compound microscope, when identifying.

Results and discussion

Increased picking efficiency

Figure 1 illustrates the difference between picking unstained and stained samples under the stereomicroscope. Photographs were taken at the same magnification with the same gradient of colours and amplification. In the stained samples, background sediment and unchitinized organisms appeared lighter and the chironomid head capsule was more apparent than in the unstained sediment where colours were more uniform, making the head capsule less visible. In the unstained samples, it was sometimes difficult to distinguish between a head capsule and other sediment particles or unchitinized organisms. Chironomid head capsules often float on the water surface in the Bogorov tray. In the stained sample, the floating chironomids were easier to find because the blue colour attracted attention while picking.

Identification

Under the microscope (200× magnification), the different parts of the head capsule had sharper contrast

Table 1 Sediment samples used for analysis

Country/location	Lake name	Sediment type	Age of sediment used
Switzerland			
47°11′N; 8°3′E	Egelsee	Highly organic	8,000 years BP
46°37′N; 7°28′E	Seebergsee	Organic and anoxic	25 years
46°19′N; 17°10′E	Unnamed Pond	Organic	Surface sediment
Germany			
52°25′N; 13°11′E	Rehwiese	Organic	Alleröd (12800-12791 BP)
52°25′N; 13°11′E	Rehwiese	Organic	Younger Dryas (12228-12219 BP)
Canada			
81°21′N; 69°32′W	LML	Inorganic	Surface sediment
82°30′N; 62°20′W	North Lake	Inorganic	Surface sediment

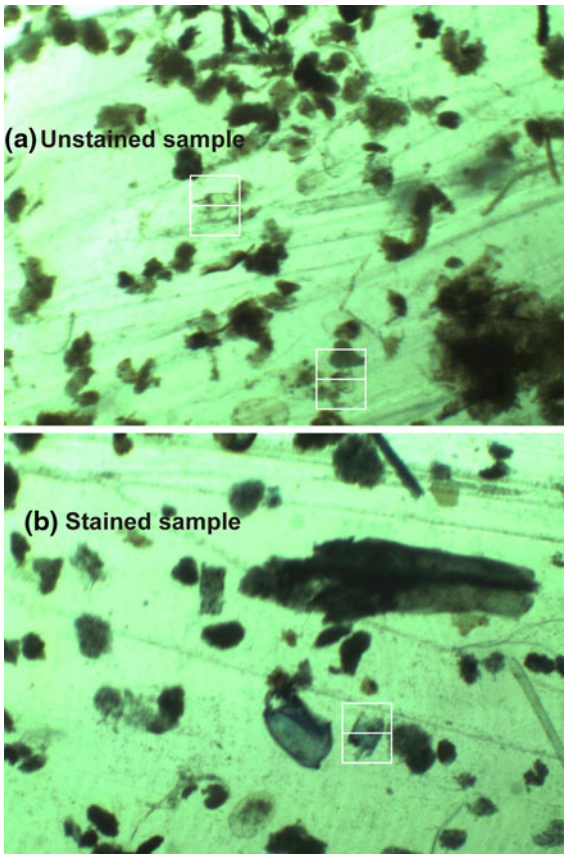

Fig. 1 Head capsules in a Bogorov tray in an (**a**) an unstained sample and in (**b**) a stained sample. The same image processing treatment was applied to both pictures. The *squares* and the *white lines* are the same size

in the stained samples. The mentum and plates remained brown/black while the head capsule was blue (Fig. 2). Note also that the head capsules from unstained samples were the clearest that were found, while the contrast of head capsules was almost always as sharp as that presented by stained samples.

At higher magnification (400×), parts of the head capsules that are sometimes hard to distinguish, such as ventromental plates in Orthocladiinae, pores on Tanypodinae, and striations on ventromental plates of Chironominae (Fig. 3), became more obvious. This higher contrast might facilitate identification, perhaps leading to greater taxonomic resolution.

Taxa in stained versus unstained samples

Percentages of taxa in stained and unstained samples were compared (Fig. 4a). Only one taxon, *Tanytarsus* without a spur on the antenna, was slightly underrepresented in one sample from surface sediments of one Swiss lake. Because this taxon name is used when no mandible is present (mandibles being used to distinguish between taxa of Tanytarsini), the difference in percentages in stained and unstained samples might arise because fewer Tanytarsini head capsules had mandibles in the stained sample. The authors do not think that this is related to the use of stain. In general, percentages of taxa found in stained and unstained samples were similar, within 5% variation.

The number of taxa in stained and unstained samples was also similar (Fig. 4b). In three samples, one taxon was found in one sample type, but was not recorded in the other sample from the same lake. However, percentages of these taxa were <2%.

In three samples (unnamed Pond, Rehwiese Allerröd, North Lake) one head capsule of *Polypedilum*, one of *Sergentia* and half a head capsule of *Heterotrissocladius subpilosus*-group, respectively, were found unstained. This represents <1% of the head capsules found in one sample. These head capsules had a worn mentum and were very pale. It is possible that they contained less chitin than other stained chironomids.

Other observations were made while picking/identifying, but were not quantified:

- third instar head capsules were stained but paler than the fourth instar head capsules. This was not illustrated here because the quality of the camera did not allow for accurate comparison of the colours. However, this difference was evident while identifying the head capsules. This difference might be due to a lower quantity of chitin in 3rd instars compared to 4th instars (Iovino 1975).
- The number of non-chironomid organisms mounted on slides was lower in stained samples, perhaps because the stained chitinuous remains of chironomids are differentiated from the unstained remains of many other organisms. This would benefit less-experienced lab assistants who sometimes mistakenly mount non-chironomid material, which increases the picking and slide preparation time.

Time required to pick a sample

The time needed to pick unstained samples was 4–8 h. Picking time was reduced by 2–4 h when

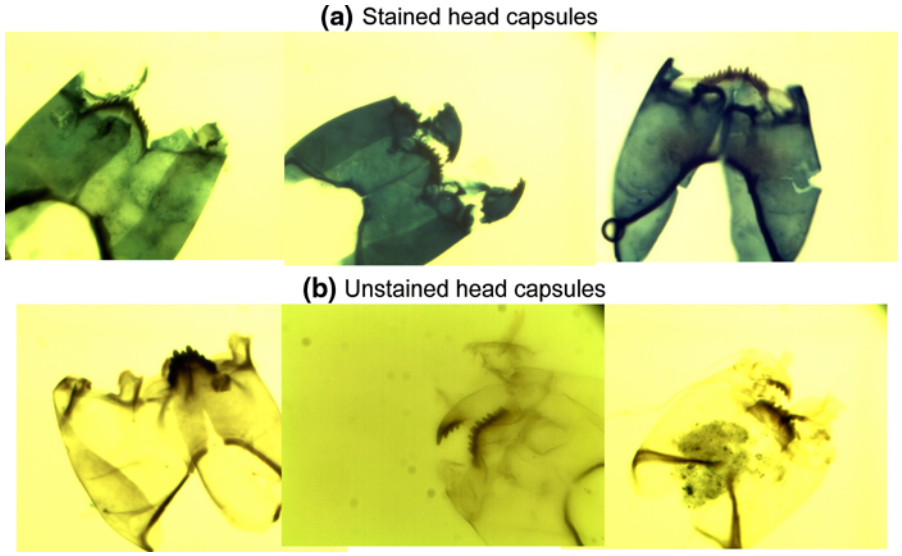

Fig. 2 (**a**) Stained and (**b**) unstained head capsules of various taxa. The head capsules were photographed at ×200 magnification

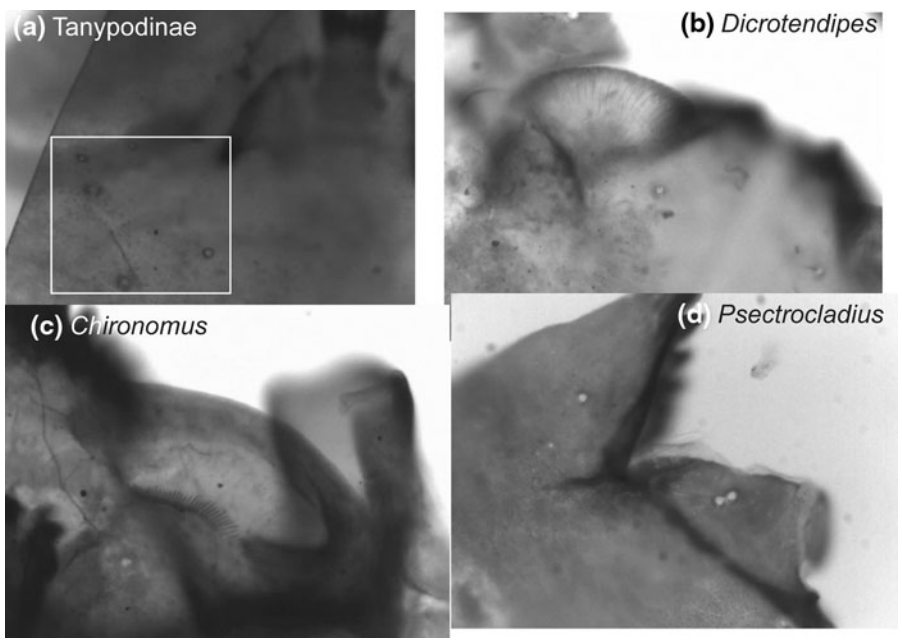

Fig. 3 Staining improves the visibility of the ventromental plate and hair on the plate in (**d**), striations on ventromental plates in (**b**) and (**c**) and the pores in (**a**) (magnification ×400)

samples were stained. The time needed to identify the chironomids was not measured, but we surmise that, due to the increase in contrast of stained head capsules, identification should also be faster.

Conclusions

Staining lake sediment samples with cotton blue improves the efficiency of picking chironomid head

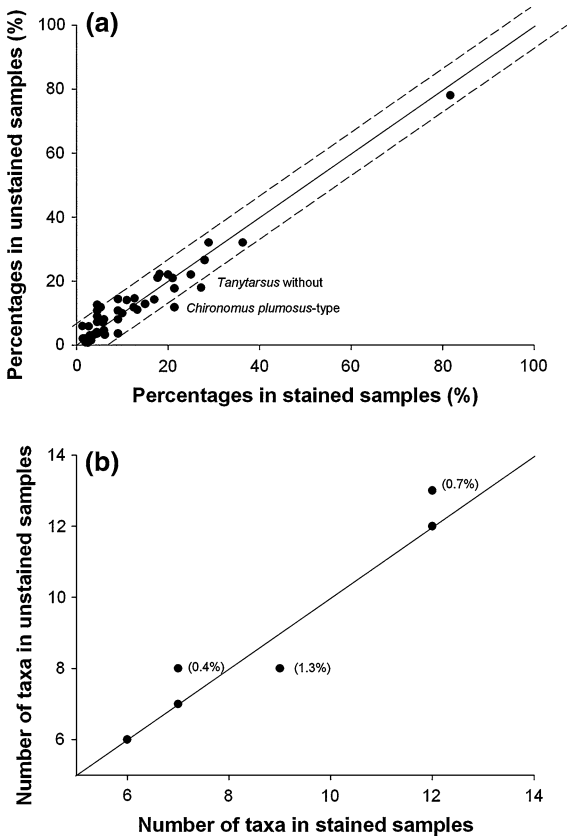

Fig. 4 Comparison of picking efficiency between stained and unstained samples. (**a**) Percentage of taxa found in stained and unstained samples. The line is the 1:1 ratio, the *dotted lines* are the 5% deviation from the 1:1 line. Taxa located outside this range (such as *Tanytarsus* without spur on the antenna) are over or under-represented. (**b**) Number of taxa found in stained and unstained samples. The line is the 1:1 ratio. The numbers in *brackets* are the percentage abundance of the taxon in the sample

capsules. It is likely that staining also improves efficiency of identification. Efficiency is improved because:

(1) head capsules are more visible in the picking tray
(2) few non-chironomid specimens (e.g. seeds) are mounted on slides because they are not stained
(3) identification is not compromised by staining the head capsules because the contrast between different parts of the head capsule (e.g. the mentum and the ventromental plates) increases, which aids identification, thereby improving taxonomic resolution.

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