

# Using morphological characters of subfossil daphniid postabdominal claws to improve taxonomic resolution within species complexes

Jennifer B. Korosi · Adam Jeziorski ·  
John P. Smol

Received: 11 March 2011 / Accepted: 23 May 2011 / Published online: 15 June 2011  
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**Abstract** *Daphnia* subfossils from lake sediments are useful for exploring the impacts of environmental stressors on aquatic ecosystems. Unfortunately, taxonomic resolution of *Daphnia* remains is coarse, as only a small portion of the animal is preserved, and so the identification of daphniid subfossils typically relies upon postabdominal claws. Daphniid claws can be assigned to one of two species complexes: *D. longispina* or *D. pulex*. Both complexes contain species with differing environmental optima, and therefore improved taxonomic resolution of subfossil daphniid claws would aid paleolimnological analyses. To identify morphological features that may be used to help differentiate between species within complexes, we used species presence/absence data from net tows to select lakes in central Ontario (Canada) containing only a single species from a particular complex, then used remains preserved in

surface sediments of these lakes to isolate four *Daphnia* species: *D. ambigua* and *D. mendotae* from the *D. longispina* complex, and *D. pulicaria* and *D. catawba* from the *D. pulex* complex. Our analyses demonstrate that, within the *D. longispina* complex, postabdominal claw length (PCL) and spinule length can be used to distinguish *D. mendotae* from *D. ambigua*. In addition, within the *D. pulex* complex, there are differences between *D. pulicaria* and *D. catawba* in the relative lengths of the proximal and middle combs on the postabdominal claw. However, the number of stout spines on the middle comb is an unreliable character for differentiating species. Overall, our data demonstrate that greater resolution within *Daphnia* species complexes is possible using postabdominal claws; however, the process is arduous, and applicability will likely decrease with the number of taxa present.

**Keywords** *Daphnia* · Paleolimnology · Species complexes · Identification guide · Postabdominal claws

Guest editors: H. Eggermont & K. Martens / Cladocera as indicators of environmental change

J. B. Korosi (✉) · A. Jeziorski · J. P. Smol  
Paleoecological Environmental Assessment and Research  
Laboratory (PEARL), Department of Biology,  
Queen's University, Kingston, ON K7L 3N6, Canada  
e-mail: jennifer.korosi@queensu.ca

A. Jeziorski  
e-mail: adam.jeziorski@queensu.ca

J. P. Smol  
e-mail: smolj@queensu.ca

## Introduction

Cladoceran subfossils in lake sediments have been used to examine the long-term biological implications of a number of environmental stressors, including major fish kills (Amsinck et al., 2005), lakewater calcium decline (Jeziorski et al., 2008), and lake

acidification (Paterson, 1994). However, the disarticulation of Cladocera into their component parts upon death, and the taxonomic difficulties inherent in the differential preservation of species remains in lake sediments, present a challenge for the interpretation of cladoceran subfossil records. This is particularly true for species of the genus *Daphnia*, because typically identifications of daphniid subfossil remains are made using only postabdominal claws and ephippia. As a result, taxonomic resolution of sedimentary *Daphnia* remains is coarse at best, as it is not currently possible to attribute a particular postabdominal claw to an individual species.

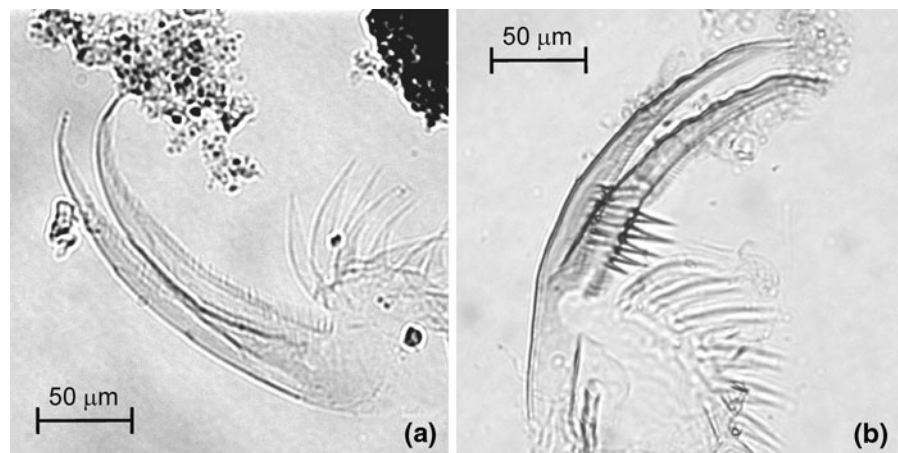
The current practice in both European and North American sedimentary cladoceran analyses is to differentiate *Daphnia* claws into two species complexes. The first complex contains species with uniform spinules along the postabdominal claw (Fig. 1a), and is referred to by many different names in the paleolimnological literature including the *Daphnia longispina* complex in European studies (Korhola, 1999; Bigler et al., 2006; Kamenik et al., 2007), and in North American studies as either the *D. rosea* complex, (Leavitt et al., 1994; Bos et al., 1999), the *D. dentifera* complex (Bredesen et al., 2002), or the *D. ambigua* complex (DeSellas et al., 2008). Here, we refer to this group as the *Daphnia longispina* complex, in order to better harmonize the European and North American literature on subfossil Cladocera. The second daphniid complex is universally referred to as the *Daphnia pulex* complex, and consists of species with stout spines on the middle comb of the postabdominal claw (Fig. 1b; Brugam &

Speziale, 1983; Bigler et al., 2006; DeSellas et al., 2008).

Ecological requirements and relative sensitivity to invertebrate and fish predation vary among *Daphnia* species (e.g. Gliwicz, 1990). Therefore, there is considerable information potentially available to paleolimnologists if a reliable method was developed for identifying *Daphnia* species based on postabdominal claws. For example, *D. mendotae* (within the *D. longispina* complex) has been shown to survive the invasion of the predatory cladoceran *Bythotrephes longimanus* into North American lakes while other smaller *Daphnia* species are lost (Lehman & Cáceres, 1997; Yan et al., 2001). The isolation of *D. mendotae* based on postabdominal claws from lake sediments would therefore be useful for paleolimnological studies examining the spread of *Bythotrephes*. In addition, in eastern Canada, *D. ambigua* (*D. longispina* complex) and *D. catawba* (*D. pulex* complex) are relatively acid-tolerant (Keller & Pitblado, 1984), and are also tolerant of low calcium (Ca) concentrations (Cairns 2010), well below the 1.5-mg/l fitness threshold identified for *D. pulex* (Ashforth & Yan, 2008). Therefore, by grouping species together into complexes, we reduce the effectiveness of cladoceran subfossils as paleolimnological indicators of environmental stressors such as lakewater acidification and aqueous Ca decline (e.g., Jeziorski et al., 2011).

Hebert & Finston (1997) showed that the number of stout spines on the middle comb of the postabdominal claw was a useful feature for separating *D. pulex* from *D. catawba*; *D. catawba* typically has 3–4 stout spines, and *D. pulex* ordinarily has 5+. This

**Fig. 1** **a** A postabdominal claw with uniform spinules representative of the *Daphnia longispina* complex, and **b** A postabdominal claw with stout spines on the middle comb representative of the *D. pulex* complex



distinction between the number of stout spines present on the middle comb has occasionally been applied to *Daphnia* subfossils (e.g. Bredesen et al., 2002; Bos & Cumming, 2003); however, there are disparities in the literature that question the validity of the number of stout spines as a diagnostic character in a paleolimnological context, when no additional diagnostic features can be used to validate identifications. For example, Paterson (1994) identified claws in the sediments of lakes in Adirondack Park (New York, USA) with 3–5 stout spines as belonging to *D. catawba*, Schwartz et al. (1985) designate *D. pulex* as having 4–9 stout spines, and examples of *D. pulex* postabdominal claws with four stout spines can be found in Hebert (1995). Furthermore, postabdominal claws have been recovered from lake sediments in south-central Ontario that clearly show four stout spines on one claw, and five stout spines on the other (Fig. 2).

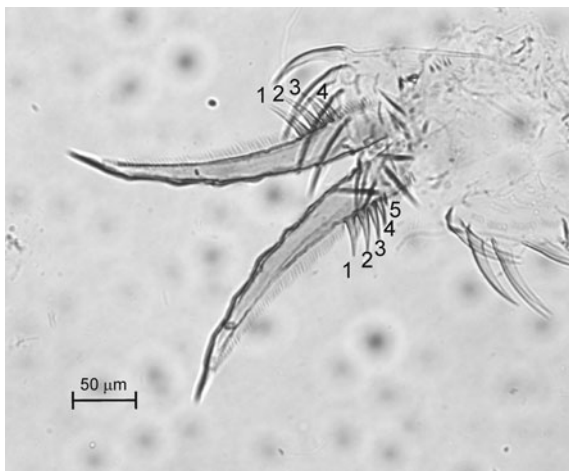
Here, our objective is to perform a detailed examination of the features of postabdominal claws from several *Daphnia* species in south-central Ontario, Canada (including claw length, width, curvature, and spine/spinule length), to determine whether subtle differences exist that can be used to aid taxonomic resolution within species complexes. A set of four lakes was selected from a broad lake survey of pelagic zooplankton conducted by

the Canadian Aquatic Invasive Species Network (CAISN) that contained only a single daphniid species from a given species complex. Subsequently, surface sediments were collected from the four lakes, with the assumption that the daphniid postabdominal claws recovered in these samples belonged to the taxon identified in the modern-day net tows. Using this method, we compare *D. ambigua* to *D. mendotae* within the *D. longispina* complex, and within the *D. pulex* complex, we compare *D. catawba* to *D. pulicaria*, a taxon that is morphologically identical to *D. pulex* and distinguished from it based only on genetics (Hebert et al., 1993) or habitat type (*D. pulex* is a strict pond dweller; Dudycha, 2004, Hebert, 1995). This exploratory study will not only evaluate whether it is possible to reliably distinguish between *Daphnia* species based on claws in lake sediments from south-central Ontario, but as differentiation of *Daphnia* claws is a concern for paleolimnologists globally, our regional findings will have much wider implications. We show that subtle differences in claw morphology do exist between *D. ambigua* and *D. mendotae*, as well as *D. pulex* and *D. catawba*, however the application of these differences to the identification of subfossil daphniid postabdominal claws will be labor-intensive, and some uncertainty in identifications will still be present.

## Materials and methods

### Lake selection and field methods

From mid-June to late August in 2005 and 2006, 311 lakes in south-central Ontario were sampled for zooplankton as part of a broad study to document the spread of the invasive species *Bythotrephes longimanus* by the Canadian Aquatic Invasive Species Network (Cairns et al., 2007). Duplicate vertical net hauls were taken from a deepwater station along the fetch of each lake using a 63  $\mu\text{m}$  mesh conical net tow, and a composite sample that contained a minimum of 250 individuals was counted and used to determine daphniid species presence/absence (Cairns 2010). From this dataset, we selected three lakes based on their relatively simple daphniid communities: Oudaze Lake, which contained only *D. mendotae* from the *D. longispina* complex and no species from the *D. pulex* complex, Dunbar Lake, which contained



**Fig. 2** A *Daphnia pulex* complex claw recovered from the surface sediments of Dunbar Lake in south-central Ontario that has four stout spines on one claw, and five stout spines on the other

**Table 1** Daphniid species presence/absence data obtained from Cairns et al. (2007) for the four study lakes

Lake	Species present ( <i>Daphnia longispina</i> complex)	Species present ( <i>Daphnia pulex</i> complex)
Young	<i>D. mendotae</i> <sup>a</sup>	None
Oudaze	<i>D. mendotae</i> <sup>a</sup>	None
Dunbar	<i>D. ambigua</i> <sup>a</sup>	<i>D. catawba</i> <sup>a</sup>
Camp	<i>D. ambigua</i> <i>D. mendotae</i> <i>D. longiremis</i>	<i>D. pulicaria</i> <sup>a</sup>

<sup>a</sup> The species from each lake analyzed in this study

*D. ambigua* from the *D. longispina* complex and *D. catawba* from the *D. pulex* complex, and Camp Lake, which contained several species from the *D. longispina* complex, but only *D. pulicaria* from the *D. pulex* complex (Table 1; Fig. 3). In addition, we also selected Young Lake, as this lake is sampled regularly by the Dorset Environmental Science Centre (Ontario Ministry of the Environment) and currently only contains *D. mendotae*.

In summer 2007, sediment cores were collected from the deepest basins of Young, Dunbar, and Oudaze lakes using a Glew (1989) gravity corer (Jeziorski et al., 2011). A Glew (1988) vertical extruder was used to extract the top 0.25 cm of sediment from each core, which in this region typically represents the last 0–3 years of sediment accumulation (e.g., Mills et al., 2009). A surface sediment sample was collected in an identical manner from Camp Lake in September 2010. To account for the 4-year gap between modern zooplankton sampling in 2006 and sediment collection in 2010, both the 0–0.25 and 0.25–0.5 cm sediment intervals were analyzed to ensure that the material included the 2006 sampling season.

#### Laboratory methods

Approximately, 1.0 g of wet sediment was deflocculated in 10% KOH for ~30 min at 70–80°C, following the general techniques outlined in Korhola & Rautio (2001). The solution was then passed through a 37 µm sieve, and the residue retained on the sieve was transferred to a vial with deionized water. Safranin–glycerol solution was added to color

the remains, and 10% ethanol was added to prevent fungal growth. Slides were then mounted using glycerin jelly.

For *D. catawba* (Dunbar Lake), *D. mendotae* (Young and Oudaze lakes combined), and *D. pulicaria* (Camp Lake), a minimum of 50 claws were recovered and analyzed at 400× magnification on a Leica DMRB microscope using bright field optics. We selected 50 claws per species consistent with previous paleolimnological investigations into daphniid postabdominal claw length (PCL, Manca et al., 2000; Korosi et al., 2008, 2010). Only 46 claws were recovered for *D. ambigua* from Dunbar Lake. On all claws, the PCL, postabdominal claw width (PCW), spinule length (SL), and two different angles ( $\emptyset_1$  and  $\emptyset_2$ ) were measured (Fig. 4a) using Northern Eclipse Image Analysis Software version 6 (Empix Imaging Inc.) For *D. catawba* and *D. pulicaria*, comb lengths (CL) and spine/spinule length (SL) for the proximal, middle, and distal portions of the claw were also measured (Fig. 4b), and the number of stout spines present on the middle comb was recorded.

#### Statistical analysis

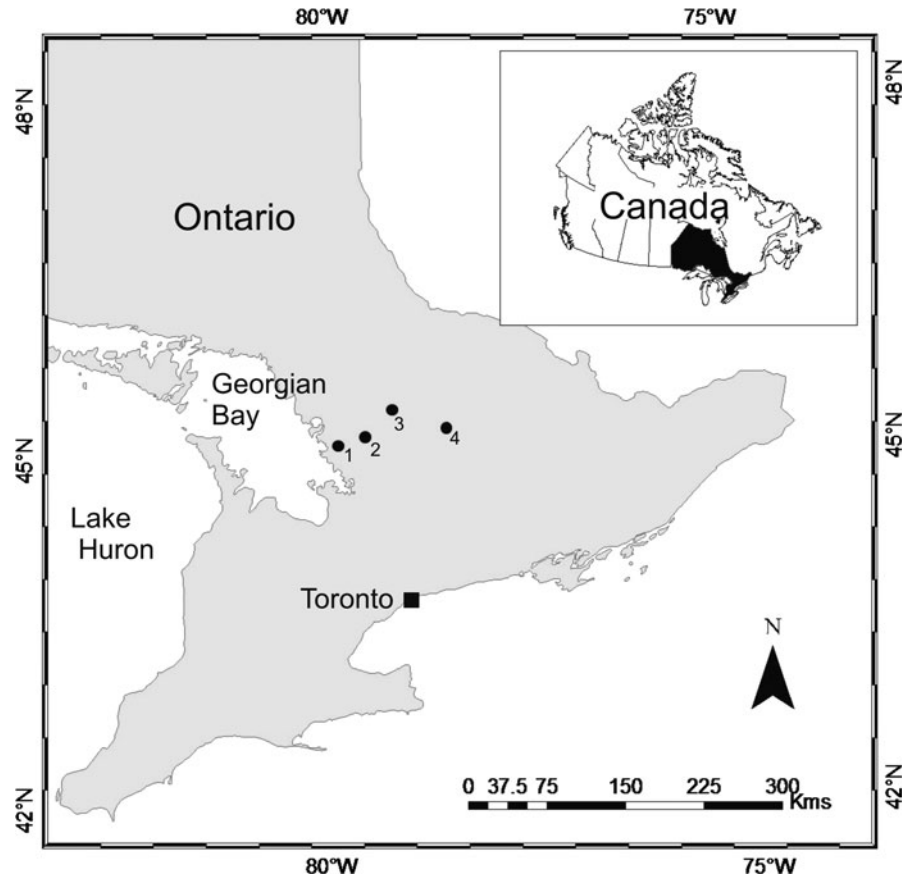
To determine whether *D. ambigua* could be separated from *D. mendotae*, and *D. pulicaria* from *D. catawba*, a nonparametric classification and regression-tree (CART) analysis was conducted using the *rpart* package (Therneau et al., 2009) for the R software environment (R Development Core Team, 2011) to assess the minimum number of claw traits that would allow for robust differentiation between species. This recursive approach has previously been successfully applied in a paleolimnological context to differentiate between morphologically similar pollen grains (Lindbladh et al., 2002; Barton et al., 2010).

## Results

#### *Daphnia longispina* complex

Postabdominal claw lengths (PCL) significantly differed between *D. ambigua* and *D. mendotae* samples (*t* test,  $P < 0.0001$ ). The size distribution of *D. mendotae* was skewed toward higher values compared to *D. ambigua*, which never exceeded a length of 150.0 µm (Table 2; Fig. 5). To estimate within-

**Fig. 3** A map of south-central Ontario showing the locations of the four study lakes (black circles), Dunbar Lake (1), Oudaze Lake (2), Young Lake (3), and Camp Lake (4). Inset shows the location of Ontario within Canada



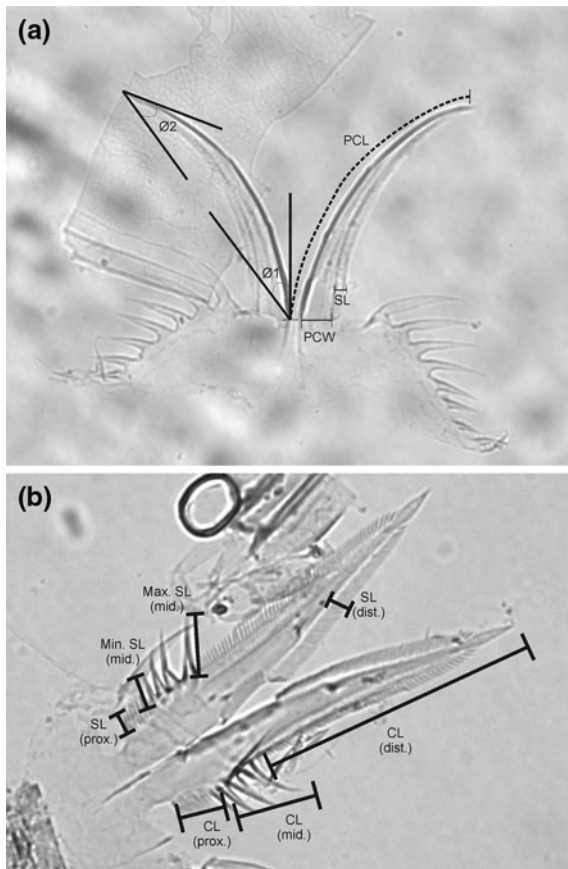
species variation, as well as inter-specific variation, we also compared *D. mendotae* claws recovered from Oudaze Lake to *D. mendotae* claws from Young Lake. Although the average length of *D. mendotae* claws from Oudaze Lake was significantly longer than *D. mendotae* claws from Young Lake (*t* test,  $P = 0.007$ ; Fig. 6), the smaller *D. mendotae* claws from Young Lake were still significantly longer than *D. ambigua* claws (*t* test,  $P = 0.0001$ ; Fig. 6).

The classification tree produced by CART determined that, of the measured variables, *D. ambigua* and *D. mendotae* claws were best separated by postabdominal claw length (PCL) and spinule length (SL; Fig. 7a). Of the claws that were greater in length than 133.3  $\mu\text{m}$ , only three were from *D. ambigua*, while 34 were from *D. mendotae* (Fig. 7a). Claws < 133.3  $\mu\text{m}$  long were further divided based on SL, where amongst the claws with  $\text{SL} \geq 4.3 \mu\text{m}$ , 32 were from *D. ambigua* and four were from *D. mendotae*. Of claws with  $\text{SL} < 4.3 \mu\text{m}$  and  $\text{PCL} < 93.1 \mu\text{m}$ , ten were from *D. ambigua* and six were from *D. mendotae*, and

for claws with  $\text{SL} < 4.3$  and  $\text{PCL} > 93.1 \mu\text{m}$ , six were from *D. mendotae* and one was from *D. ambigua*. In general, *D. ambigua* claws were shorter with longer spinules than *D. mendotae* claws, although a degree of uncertainty is present.

#### *Daphnia pulex* complex

No significant difference in claw morphology was observed between *D. pulicaria* claws in Camp Lake recovered from the 0–0.25 and 0.25–0.5 cm sediment intervals (ANOSIM,  $P > 0.05$ ), therefore all claws from Camp Lake were grouped together for comparison with *D. catawba* claws from Dunbar Lake. *D. catawba* claws had 3–4 stout spines on the middle comb, and *D. pulicaria* had 3–7, but the majority of claws recovered for both species had four stout spines (Fig. 8). *D. pulicaria* claws were significantly longer than *D. catawba* (*t* test,  $P = 0.001$ ); however, there was more overlap in size than compared with *D. ambigua* and *D. mendotae* (Table 3; Fig. 9).



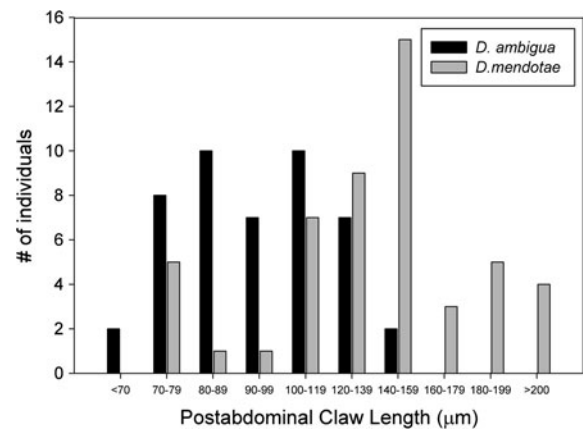
**Fig. 4** **a** Measurements used to characterize *Daphnia* postabdominal claws. *PCL* postabdominal claw length, *PCW* postabdominal claw width, *SL* spinule length. A claw belonging to the *D. longispina* complex is shown here, but these measurements were also taken for claws in the *D. pulex* complex. **b** Additional measurements taken for claws belonging to the *D. pulex* complex, including proximal (*prox.*) comb length (*CL*) and spinule length (*SL*), distal (*dist.*) *CL* and *SL*, and middle (*mid.*) *CL* and spine length (*SL<sub>min</sub>* and *SL<sub>max</sub>*)

Neither postabdominal claw length (*PCL*) nor the number of stout spines was identified as a predictor of species identity by the CART analysis. Instead, the best split was based on the length of the middle comb of the claw (*CL<sub>mid</sub>*) where the stout spines are located (Fig. 7b). Of claws with *CL<sub>mid</sub>* > 29.3  $\mu\text{m}$ , 26 were from *D. pulicaria* and three were from *D. catawba* (Fig. 7b). Claws with *CL<sub>mid</sub>* < 29.3  $\mu\text{m}$  were further split based on the length of the proximal comb of the claw (*CL<sub>prox</sub>*), and 31 claws with *CL<sub>prox</sub>*  $\geq$  13.2  $\mu\text{m}$  belonged to *D. catawba* while nine were *D. pulicaria*. This portion of the regression tree was split again based on *CL<sub>mid</sub>*, where ten claws with

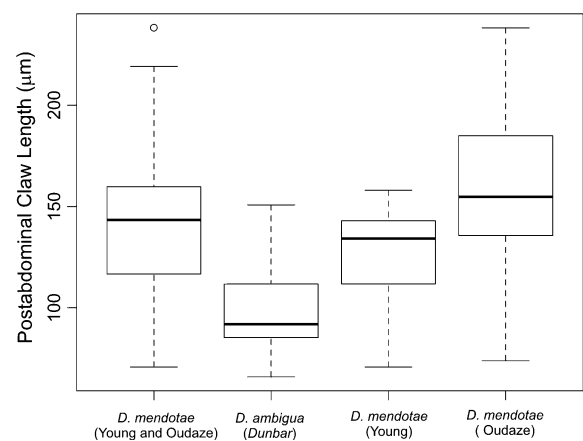
**Table 2** Descriptive statistics for the specimens analyzed from the *Daphnia longispina* complex

	<i>PCL</i> ( $\mu\text{m}$ )	<i>PCW</i> ( $\mu\text{m}$ )	<i>SL</i> ( $\mu\text{m}$ )	$\text{Ø}_1$ ( $^\circ$ )	$\text{Ø}_2$ ( $^\circ$ )
<i>D. mendotae</i> ( <i>n</i> = 50)					
Min	70.8	6.6	2.2	0	0
Max	238.2	27.0	8.9	40.0	138.3
Mean	142.4	15.4	5.4	25.0	43.4
<i>D. ambigua</i> ( <i>n</i> = 46)					
Min	65.9	6.2	3.0	21.0	30.0
Max	150.8	21.8	12.9	44.1	128.8
Mean	98.4	12.0	5.1	30.8	51.1

*PCL* postabdominal claw length, *PCW* postabdominal claw width, *SL* spinule length

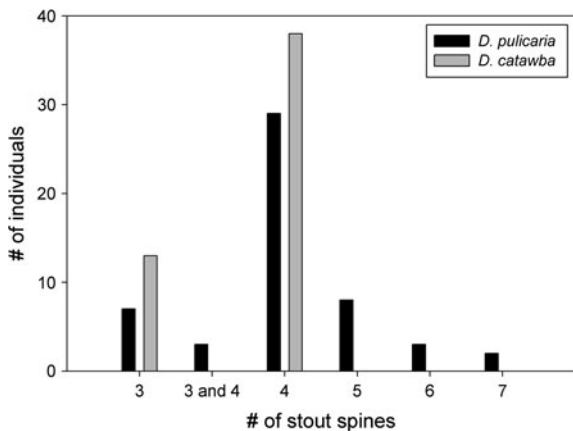
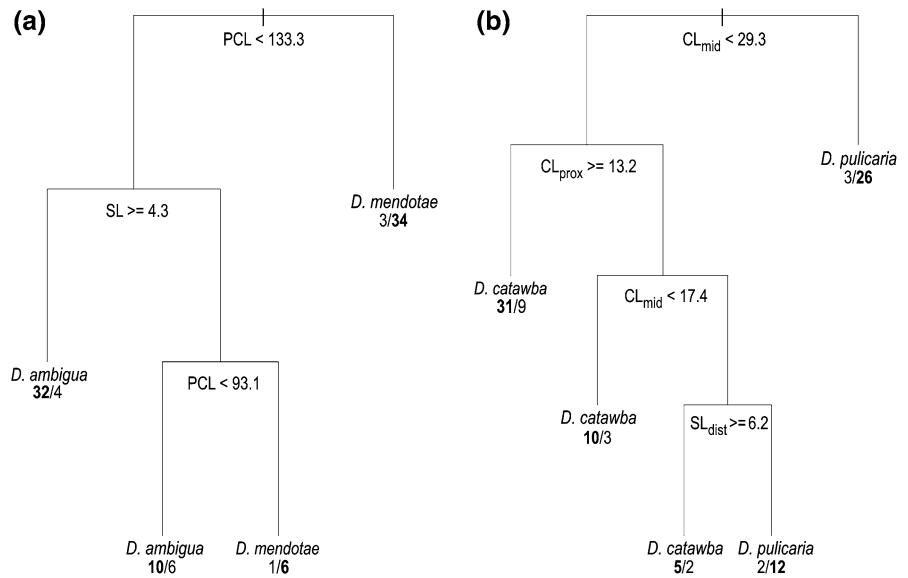


**Fig. 5** Distribution of postabdominal claw lengths (*PCL*) for *Daphnia ambigua* and *D. mendotae*



**Fig. 6** Boxplots comparing postabdominal claw length between *Daphnia ambigua* in Dunbar Lake, and *D. mendotae* recovered from Young, Oudaze, and Young + Oudaze lakes

**Fig. 7** Classification tree produced by (CART) analysis of **a** *Daphnia ambigua* and *D. mendotae* claws, and **b** *D. catawba* and *D. pulicaria* claws. Measurements are in  $\mu\text{m}$ . The numbers below species name refer to the number of claws correctly classified (**bold**), and the number of claws incorrectly classified (*not bold*)



**Fig. 8** A histogram showing the number of stout spines on the middle comb of *Daphnia pulicaria* and *D. catawba* postabdominal claws

$CL_{prox} < 13.2 \mu\text{m}$  and  $CL_{mid} < 17.4 \mu\text{m}$  were from *D. catawba* and three were from *D. pulicaria*. The final split was based on the spinule length on the distal comb of the claw ( $SL_{dist}$ ), where 12 *D. pulicaria* claws and two *D. catawba* claws had  $PL_{dist} < 6.2 \mu\text{m}$ , and five *D. catawba* and two *D. pulicaria* claws had  $SL_{dist} \geq 6.2 \mu\text{m}$ .

## Discussion

Subtle differences in claw morphology were observed between *Daphnia ambigua* and *D. mendotae* in the

*D. longispina* complex, as well as *D. pulicaria* and *D. catawba* in the *D. pulex* complex (summarized in Table 4). The application of these features as a taxonomic tool for subfossil Cladocera analyses, however, will be considerably more cumbersome than the traditional method of assigning complexes, and a small degree of uncertainty appears to be unavoidable.

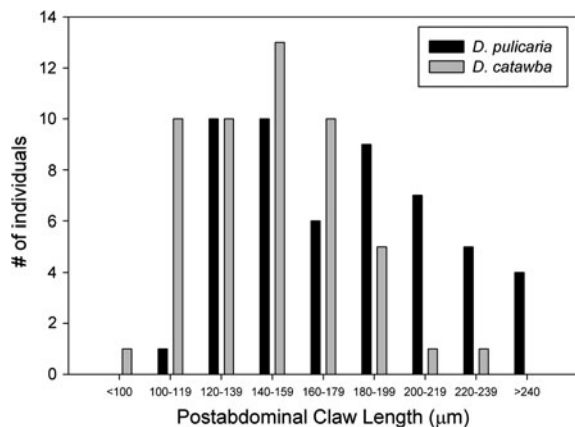
### *Daphnia longispina* complex

As expected, a significant difference in postabdominal claw length (PCL) was observed between the two species, since *D. mendotae* is the largest daphniid species found in central Ontario (1.2–2.8 mm), while *D. ambigua* is the smallest (max. size 1.3 mm; Hebert, 1995; Witty, 2004). More than half of *D. mendotae* claws recovered had  $PCL > 133 \mu\text{m}$ , with several claws  $> 200 \mu\text{m}$  in length. Conversely, only three *D. ambigua* claws were  $> 133 \mu\text{m}$ , and none reached a maximum size  $> 150 \mu\text{m}$ . Shifts in claw length, therefore, have the potential to be used as a crude method for identifying shifts in the dominant daphniid taxa in paleolimnological analyses for some species. For example, it has been suggested that differences in *D. longispina* complex claw length across environmental gradients likely reflect *Daphnia* species turnover (Korosi et al., 2008), and a decrease in size within the *D. longispina* complex since pre-industrial times has been interpreted as an increase in

**Table 3** Descriptive statistics for the specimens analyzed from the *Daphnia pulex* complex

<i>D. pulicaria</i> (n = 53)											
	No of stout spines	PCL (μm)	PCW (μm)	CL <sub>prox</sub> (μm)	SL <sub>prox</sub> (μm)	CL <sub>mid</sub> (μm)	SL <sub>mid</sub> (μm)	CL <sub>dist</sub> (μm)	SL <sub>dist</sub> (μm)	Ø <sub>1</sub> (°)	Ø <sub>2</sub> (°)
Min	3	102.5	9.9	8.4	4.0	14.7	5.7–14.1	70.6	3.4	5.4	5.5
Max	7	266.9	32.2	25.4	15.5	58.8	25.6–39.7	176.8	12.8	41.9	57.9
Mean	–	178.2	19.6	15.9	8.1	28.6	14.2–27.0	121.2	7.5	18.7	28.3
<i>D. catawba</i> (n = 51)											
Min	3	91.4	10.4	9.6	2.0	14.3	3.2–11.2	53.5	2.9	7.0	6.3
Max	4	221.0	23.1	21.5	12.2	30.7	23.1–34.7	152.2	12.4	38.9	85.6
Mean	–	147.2	17.0	14.6	7.2	22.0	11.7–23.3	102.9	7.2	21.5	35.0

PCL postabdominal claw length, PCW postabdominal claw width, SL spinule length (or spine length for the middle comb), CL<sub>prox</sub> and SL<sub>prox</sub> refer to proximal comb and spinule lengths, CL<sub>mid</sub> and SL<sub>mid</sub> for middle comb and spine lengths, and CL<sub>dist</sub> and SL<sub>dist</sub> for distal comb and spinule length

**Fig. 9** Distribution of postabdominal claw lengths (PCL) for *Daphnia pulicaria* and *D. catawba*

the relative abundance of *D. ambigua* related to acidification in the region (Korosi et al., 2010).

Phenotypic plasticity places limitations upon the interpretation of changes in mean postabdominal claw length as shifts among daphniid taxa, as we observed *D. mendotae* claws recovered from Young Lake to be significantly smaller than those recovered from Oudaze Lake. Various environmental factors can influence daphniid body size, and lead to the differences in size structure observed between *D. mendotae* in Young and Oudaze lakes. Higher lakewater temperatures, for example, may increase the growth rate and moulting frequency of a daphniid, but lead to decreased feeding efficiency and smaller body size at maturity (Moore et al., 1996). Size-

selective predation can also affect intra-species size variability, and invertebrate predators like *Bythotrephes* often select for larger *Daphnia* individuals (Manca et al., 2000; Yan et al., 2001) while fish predation selects for smaller daphniids (Beckerman et al., 2010). *Daphnia* that are under predation pressure by both invertebrates and fish may have a faster growth rate (to minimize vulnerability to invertebrates), but a smaller body size at maturity (Lynch, 1977). In Oudaze and Young lakes, *D. mendotae* claws belonging to the smaller size class tended to have shorter spinule lengths (SL) than *D. ambigua*; therefore, an approach that combines PCL and SL measurements may be more effective at detecting species shifts within the *D. longispina* complex in lake sedimentary records. No useful taxonomic information was provided by PCW or curvature, and performing these measurements is likely not a worthwhile time investment.

Within central Ontario, three additional members of the *D. longispina* complex exist that are each intermediate in size between *D. ambigua* and *D. mendotae* (*D. dubia*, 1.1–1.9 mm; *D. longiremus*, 0.6–2.4 mm, and *D. dentifera*, 0.9–2.2 mm; Witty 2004; Hebert, 1995). Future analyses could examine the qualitative features and size distributions of these taxa relative to *D. ambigua* and *D. mendotae*. Still, the results of this exploratory study are promising, given the importance of these two taxa for addressing current research questions in central Ontario relating to calcium decline (*D. ambigua* is tolerant of low [Ca]; Cairns 2010) and spread of *Bythotrephes longimanus* (*D. mendotae* is



**Table 4** The main morphological characters of *Daphnia* postabdominal claws recovered from lake sediments in south-central Ontario that may be useful for differentiating between*D. catawba* and *D. pulicaria* within the *D. pulex* complex, and *D. ambigua* and *D. mendotae* within the *D. longispina* complex

Character	<i>D. pulex</i> complex		<i>D. longispina</i> complex	
	<i>D. catawba</i>	<i>D. pulicaria</i>	<i>D. ambigua</i>	<i>D. mendotae</i>
Postabdominal claw length (PCL)	Long (mean = 147 $\mu\text{m}$ )	Long (mean = 178 $\mu\text{m}$ )	Short <sup>a</sup> (mean = 98 $\mu\text{m}$ )	Long <sup>a</sup> (mean = 142 $\mu\text{m}$ )
Spinule length (SL)	Shorter <sup>a</sup> (mean = 7.2 $\mu\text{m}$ for distal comb)	Longer <sup>a</sup> (mean = 7.5 $\mu\text{m}$ for distal comb)	Longer <sup>a</sup> (SL/PCL = 0.05)	Shorter <sup>a</sup> (SL/PCL = 0.04)
No stout spines on middle comb	3–4	3–7	n/a	n/a
Proximal:Middle comb length (CL <sub>prox</sub> :CL <sub>mid</sub> )	Longer CL <sub>prox</sub> relative to CL <sub>mid</sub> <sup>a</sup> (CL <sub>prox</sub> :CL <sub>mid</sub> = 0.67)	Shorter CL <sub>prox</sub> relative to CL <sub>mid</sub> <sup>a</sup> (CL <sub>prox</sub> :CL <sub>mid</sub> = 0.56)	n/a	n/a

<sup>a</sup> Diagnostic characteristics that were identified in CART analysis as important for differentiating between *D. pulicaria* and *D. catawba*, or *D. ambigua* and *D. mendotae*

able to co-occur with *Bythotrephes*; Yan et al., 2001). Measuring claw and spinule lengths from lake sediments has the potential to reveal biologically relevant species trends within the *Daphnia* community unavailable from the species complex dichotomy alone, presenting an opportunity for resolving *Daphnia* species identity using subfossils.

#### *Daphnia pulex* complex

The number of stout spines on the middle comb of the postabdominal claw has been previously identified as a useful feature for differentiating between species of the *D. pulex* complex using whole specimens (Hebert & Finston, 1997), as well as in subfossil analyses using only the postabdominal claw (Bos & Cumming, 2003). In general, *D. catawba* and *D. minnehaha* are identified as having 3–4 stout spines, and *D. pulex* and *D. pulicaria* as having five or more (Hebert & Finston, 1997). While our data do support the assumption that *D. catawba* generally have 3–4 stout spines, *D. pulicaria* were frequently observed in this study to have <5 stout spines. Therefore, rather than a definitive diagnostic character, the number of stout spines at best provides an additional clue for species identification. This is useful for identification based upon entire animals, where a number of other morphological characteristics can be considered including the length of the tail spine, abdominal processes, and degree of pubescence (Hebert, 1995). However, application of this distinction to

paleolimnological studies, without the benefit of additional taxonomic features, may underestimate the prevalence of *D. pulex/pulicaria*.

The postabdominal claws of *D. pulicaria* were significantly longer than those of *D. catawba*; however, the overlap in size between the two species prevent the use of postabdominal claw length (PCL) as a reliable taxonomic tool. The CART analysis identified the lengths of the middle and proximate combs of the claw as useful for distinguishing between the two species: in *D. pulicaria* the length of the middle comb of the claw is longer, whereas in *D. catawba* the proximal comb is longer. An indication of useful taxonomic differences exists, then, with respect to the relative lengths of the proximal, middle, and distal combs on the postabdominal claw, rather than postabdominal claw length.

There are a number of inherent assumptions made in our methodological approach to identifying useful features to distinguish between daphniid postabdominal claws. We assume, first, that *Daphnia* claws recovered from one lake are representative of the range of phenotypic plasticity displayed by that daphniid species over the entire region. Still, the comparison of *D. mendotae* claws from Young and Oudaze lakes demonstrated that although differences in size structure can exist within a single species between lakes, these differences were more subtle than the differences between species. Secondly, we assume that samples collected from modern-day point sample net tows are comparable to samples recovered

from surface sediments that integrate material deposited over several years (typically 0–3 years). Realistically, however, differences in species seasonal and inter-annual abundances that would be captured by the sediment record are potentially missed in the modern samples. Still, we report differences between samples/daphniid species and identify morphological characters that can be used to better differentiate *Daphnia* based on subfossil remains recovered from lake sediments. To build on the ideas presented in this study, we recommend analysis of postabdominal claws from live animals recovered in net tows, where fewer opportunities for misidentifications exist. A large sample size taken from multiple lakes will ensure that results are representative of phenotypic diversity across a given region.

Although the approach we outline here is tedious and labor-intensive, differentiating between *D. pulicaria* and *D. catawba* postabdominal claws may be a useful technique for certain research questions, and warrants further investigation. *D. catawba* is an acid-tolerant taxon that can thrive in low-Ca lakes (Cairns 2010), and is one of the most common daphniid taxa of the naturally acidic, low-Ca lakes of the Canadian Shield (Hebert & Finston, 1997). *D. catawba* has been observed to replace *D. pulicaria* in Plastic Lake (Muskoka, Ontario) when Ca concentrations declined (N. Yan, York University, personal communication), and Jeziorski et al. (2011) identified coarse species resolution and differential species tolerances to low [Ca] to be detrimental to paleolimnological investigations on the response of daphniids to aqueous [Ca] decline. Furthermore, in areas such as Nova Scotia (Canada), where no long-term zooplankton monitoring datasets exist, our current understanding of how keystone daphniid communities have changed over time in response to acidification-related stressors relies solely on paleolimnological data (Korosi & Smol, 2011). The community dynamics within the *D. pulex* complex, then, are important to our understanding of how lakes have changed since pre-industrial times in North America.

## Conclusions

The purpose of this exploratory study was to identify taxonomic differences in *Daphnia* postabdominal

claws recovered from the sediments of softwater lakes in south-central Ontario (Canada) that could be useful for differentiating daphniid subfossil remains. Within the *D. longispina* complex, potential taxonomically useful differences exist in postabdominal claw length (PCL) and spinule length (SL) between *D. ambigua* and *D. mendotae*. *D. mendotae* are larger than *D. ambigua* and also have shorter spinule lengths. In our study region of south-central Ontario, three additional members of the *D. longispina* complex are present (*D. dubia*, *D. longiremus*, and *D. dentifera*), but were excluded from our analysis. It is unknown how the claw morphologies of these three species compare with those of *D. ambigua* and *D. mendotae*; however, as they are intermediate in size, it is likely they would complicate the use of PCL and SL measurements as a taxonomic tool. Adding further complexity is the potential for *D. mendotae* to hybridize with *D. dentifera* (Taylor et al., 1996), as even when identifying whole animals, species boundaries are not entirely distinct.

The number of stout spines present on the middle comb of a postabdominal claw is an unreliable characteristic to distinguish between species in the *D. pulex* complex using subfossil remains. Instead, this feature is more useful for identifying entire animals, where additional taxonomic features are also present to improve confidence in species identification. If species resolution is critical to a research question, there may be potential value in measuring the relative lengths of the proximal, middle, and distal combs of postabdominal claws (although opportunities for misidentifications would still be present).

Cladoceran subfossils are being utilized in a growing number of paleolimnological applications. Therefore, any possible advances in the taxonomic resolution of daphniid subfossils will allow researchers to address increasingly nuanced questions, and ultimately help to improve our understanding of how lakes change over time in response to complex multiple environmental stressors.

**Acknowledgments** We thank Angelo Sorce and Kris Hadley for participation in the field work, as well as Allegra Cairns and Norman Yan of York University, and the rest of the CAISN sampling team, for providing modern-day daphniid species presence/absence data. We also thank two anonymous reviewers who improved the quality of the manuscript. This project was funded by NSERC grants to JPS and JBK, and an Ontario Premier's Discovery Award to JPS.

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