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# Efficacy of Morphological Characters for Distinguishing Nymphs of *Epitheca Cynosura* and *Epitheca Spinigera* (Odonata: Corduliidae) in Wisconsin


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# EFFICACY OF MORPHOLOGICAL CHARACTERS FOR DISTINGUISHING NYMPHS OF *EPITHECA CYNOSURA* AND *EPITHECA SPINIGERA* (ODONATA: CORDULIIDAE) IN WISCONSIN

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## ABSTRACT

Attempts to distinguish exuviae and last-instar nymphs of *Epitheca cynosura* (Say) and *Epitheca spinigera* (Selys) (Odonata: Corduliidae) using lateral spine characters have proven to be unreliable, and recent use of setae counts on only one side of the prementum or one labial palp have led to confusion because these structures often hold unequal numbers of setae on the two sides of the same specimen. Based on exuviae of 67 reared *E. cynosura* and 55 reared *E. spinigera* from lakes throughout Wisconsin, we tested the efficacy of previously used character states for distinguishing these species and searched for new characters to improve the reliability of regional keys. The most reliable diagnostic character was the combined number of setae on both sides of the prementum and on both labial palps (≤ 35 – *E. cynosura*; ≥ 36 – *E. spinigera*), which correctly determined 96% of our specimens. For the small percentage of specimens that lie in the region of overlap in total setae number, we found that total exuviae length, cerci ÷ epiproct ratios of females, tubercle distance ÷ epiproct ratios of males, and the shape of the dorsal hook on segment 8 could be used to strengthen determinations.

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Despite numerous revisions (Muttkowski 1911, 1915; Davis 1933; Kormondy 1959; Tennessen 1973), the difficult genus *Epitheca* (Odonata: Corduliidae) has caused much confusion in North America (May 1995). This genus is often referred to as *Tetragoneuria* by workers who relegate *Epitheca princeps* Hagen to the genus *Epicordulia* (see Walker 1966). Confusion about this genus has encompassed both the naming of species, with only half of the 20 names that have been referred to the genus still widely accepted today, and discriminating among species in both the adult (Donnelly 1992, 2001; Needham et al. 2000) and nymphal stages, of which the latter are our current focus. Four currently accepted species of *Epitheca* are known in Wisconsin, our focal region of study. *E. princeps* and *E. canis* (McLachlan) are readily distinguished as last-instar nymphs and exuviae. However, efforts to distinguish between the last-instar nymphs and exuviae of *E. cynosura* (Say) and *E. spinigera* (Selys) have had a long and vexing history.

Referring to *E. cynosura* and *E. spinigera*, Walker (1913) remarked that, "A careful comparison was made between the exuviae of these two species, but no differences could be detected between them, except that in *spinigera* the lateral abdominal appendages average slightly longer than those of *cynosura*. This difference, however, does not appear to be constant." In contrast

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to Needham's (1901) use of the divergence of the lateral spines of abdominal segment 9 to separate these species, Walker (1913) reported considerable variation in this character among individuals of the same species. Despite this finding, Needham and Heywood (1929) used divergence of abdominal segment 9 lateral spines as the single diagnostic character for separating *E. cynosura* from *E. spinigera* in their key to nymphs of *Tetragoneuria*. Garman (1927) found the nymphs of the species of *Tetragoneuria* in Connecticut to be so similar that he did not attempt to construct a key for their separation. In their key to nymphs of North American Anisoptera, Wright and Peterson (1944) noted that very little dependence can be placed on nymphal determinations in the genus *Tetragoneuria*. However, Needham and Westfall (1955) again used the divergence of the lateral spines on abdominal segment 9 in their key to separate the group that included *E. cynosura* from the group that included *E. spinigera*.

Kormondy (1959) conducted the first in-depth study of the taxonomy and systematics of *Tetragoneuria* that included the nymphs. He initiated the possible utility of counts of palpal and premental setae to distinguish nymphs of *E. cynosura* and *E. spinigera*, and noted that last instar nymphs of *E. spinigera* averaged larger than those of *E. cynosura*. However, he concluded that considerable variation in both counts of raptorial setae and in last-instar size made them unreliable characters for taxonomic purposes. In his summary remarks about these species, he stated that "no dependable morphological characters serve to separate individual last-instar larvae..." He noted that as populations, they differed significantly in a number of ways including the larger mean size of *E. spinigera*, the much higher growth rate of *E. spinigera* from the $11^{th}$ to $12^{th}$ instar, and the earlier seasonal appearance of *E. spinigera*.

Walker and Corbet (1975) used the number of premental setae (usually 10 or less for *E. cynosura*; usually 11 or more for *E. spinigera*) as the sole character to separate these species. In their diagnosis under *E. spinigera*, they reiterated that the relative length and direction of the lateral spines on segment 9 was not reliable, that overlap could be expected in both palpal and premental setae counts, and that *E. spinigera* averaged larger than *E. cynosura*, but only in part of the former's range. In their on-line key, Bright and O'Brien (1998) separated the species using the number of premental setae (usually not less than 11 for *E. spinigera*; usually not more than 10 for *E. cynosura*), followed by the number of palpal setae (usually 7-8 for *E. spinigera;* usually 6 for *E. cynosura*). Needham et al. (2000) similarly used the number of premental setae (usually 11-12), followed by the number of palpal setae (usually 7) to separate *E. spinigera* from the group containing *E. cynosura* (premental setae usually 8-10; palpal setae usually 5-6). We tried to use these keys to distinguish last-instar nymphs and exuviae of *E. cynosura* and *E. spinigera* from waters in Wisconsin, but were uncertain about many determinations. Many specimens belonging to one of these species had 11 or more setae on one side of the prementum, but 10 or fewer setae on the other side, or at least 7 setae on one labial palp, but only 6 setae on the other. For some specimens, counting premental setae led to a different determination than counting palpal setae (e.g.,10 or fewer setae on both sides of the prementum indicating *E. cynosura*, but 7 or more setae on each palp indicating *E. spinigera*).

To make firmer recommendations for separating exuviae and last-instar nymphs of these species, we reared specimens of both species from numerous lakes in Wisconsin to form a database on which a number of morphological analyses could be performed. Our objectives were to determine if either premental or palpal setae counts, or a combination of the two, would reliably distinguish these species in Wisconsin, and to search for and evaluate other characters that could be used to separate them.

## MATERIALS AND METHODS

We used only reared exuviae in our analyses to be certain of their identity. Nymphs were collected after molting to F-0 and were reared to emergence in aquaria. Teneral adults were maintained alive in small cages for several days after emergence, then were soaked overnight in acetone, dried, and stored in standard Odonata envelopes. Exuviae were placed in individual vials of 70% ethanol. Each adult/exuvia association was given a unique accession number immediately after emergence to preclude any possibility of confusing the specimens.

Our dataset was comprised of exuviae of 55 *E. spinigera* and exuviae of 67 *E. cynosura*. All of the exuviae except four were reared by one of the authors, and the first author verified all determinations and made all counts and measurements. Not all exuviae were used in all analyses. Two exuviae of *E. cynosura* and one exuvia of *E. spinigera* lacked essential mouth parts and could not be used for raptorial setae counts. Three exuviae of *E. cynosura* and one exuvia of *E. spinigera* were not measured for total length because the heads were detached or missing. One exuvia of *E. spinigera* had a twisted epiproct and was not used in analyses involving that character. Exuviae of 6 *E. spinigera* and 1 *E. cynosura* were omitted from analyses involving abdomen dimensions, and exuviae of 3 male *E. spinigera* and 1 male *E. cynosura* were omitted from analyses involving dimensions of the cerci and epiproct. All specimens are housed either in the Odonata Collection of the Wisconsin Department of Natural Resources (WDNR) in Superior, or in the private collection of KJT in Wautoma, Wisconsin.

Abdominal segments are designated by the letter "S" and the segment number (e.g., S9 = abdominal segment 9). Counts and measurements were done under magnification using either an ocular micrometer or millimeter rule, and all measurements are reported in mm. Counts of setae on both labial palps and on both sides of the prementum were done in dorsal view with the prementum pulled outward with a teasing needle. The most medially located premental setae were small, and care was taken to count all of them. Total lengths of exuviae were measured in dorsal view with a millimeter rule. We assessed the direction in which the tip of the dorsal hook on S8 pointed in lateral view relative to the long axis of the body. The left lateral spine on S9 was measured for width at the base and length in strict dorsal view with an ocular micrometer. Also measured in dorsal view were the lengths of both of the lateral spines on S8, the lengths of the margins of S8 including the spines, and the lengths of both cerci and the epiproct. For the males, the length of the epiproct from the base to the distal margin of the ante-apical tubercles was also measured. The abdomen was measured in ventral view from the base of S1 to the apex of S10 to determine length, and across the width of S6 with the sclerite lightly depressed to determine maximum width.

Statistical analyses were performed using SigmaStat statistical software (SPSS 1997) with alpha set at 0.05. We used one way ANOVA ($F$), or Kruskal-Wallis one way ANOVA on ranks ($H$), to test for differences in setae numbers and total exuviae lengths among lakes and years. Pearson product moment correlation ($r$) was used to examine the strength of correlation between setae numbers and total exuviae lengths. The Chi-square test ($\chi^2$) was used to examine differences between species in the shape of the left lateral spine on S9 in dorsal view: categories were straight (1), and slightly incurved (2). Mann-Whitney Rank Sum tests ($T$) were used to examine differences between species in abdomen shape ratios. Statistical tests were not applied to other key characters because our goal was to assess the performance of character states in potential key couplets, not to determine statistical significance.

Material examined – WISCONSIN: BAYFIELD CO.: Sawdust Lake, 18 April and 5 May 2005, UTM 15 632537E 5159021N (all coordinates NAD83/WGS84), RBD (exuviae of 11 reared *E. cynosura* and exuviae of 3 reared *E. spinigera*);

Eau Claire River, 8 May 2007, UTM 15 615079E 5128789N, RBD (exuviae of 2 reared *E. cynosura*); BURNETT CO.: Dunham Lake, 23 April 2005, UTM 15 541560E 5067888N, MSB (exuviae of 17 reared *E. cynosura*); Clam River, 26 April 2007, UTM 15 553423E 5076631N, RBD (exuviae of 1 reared *E. cynosura*); pond, Memory Lake Campground, 22 May 2005, 10 April 2006, and 22 February 2007, UTM 15 524426E 5069876N, MSB (exuviae of 22 reared *E. spinigera*); Poquette Lake, 9 April 2005, UTM 15 571908E 5073126N, MSB (exuviae of 7 reared *E. cynosura*); DOUGLAS CO.: Amnicon Lake, 19 May 2006, UTM 15 571420E 5147735N, RBD (exuviae of 1 reared *E. cynosura*); Breitzman Lake, 7 June 2002 and 12 May 2003, UTM 15 560242E 5145202N, RBD (exuviae of 4 reared *E. cynosura*); Gander Lake, 4 June 2002, UTM 15 597475E 5145108N, RBD (exuviae of 2 reared *E. cynosura*); Rush Lake, 20 April 2007, UTM 15 610833E 5149561N, RBD (exuviae of 6 reared *E. cynosura* and exuviae of 6 reared *E. spinigera*); Cut-A-Way Dam, St. Croix River, 1 June 2005 and 16 May 2006, UTM 15 593329E 5128758N, RBD (exuviae of 2 reared *E. cynosura* and exuviae of 3 reared *E. spinigera*); Upper St. Croix Lake, 20 May 2005, UTM 15 593857E 5136942N, RBD (exuviae of 8 reared *E. cynosura*); EAU CLAIRE CO.: Half Moon Lake, 13 November 2007, UTM 15 616483E 4962190N, RBD (exuviae of 2 reared *E. cynosura*); LANGLADE CO.: Crooked Lake, 14 May 2003, UTM 16 327200E 5021066N, KJT (exuviae of 1 reared *E. spinigera*); ONEIDA CO.: Tomahawk Lake, Kemp Natural Resources Center, 14 June 2004, UTM 16 291966E 5079767N, RBD (exuviae of 1 reared *E. spinigera*); RICHLAND CO.: Cruson Slough, Wisconsin River, 21 April 2007, UTM 15 724367E 4785883N, W. A. Smith (exuviae of 4 reared *E. cynosura*); WAUSHARA CO.: Bohn Lake, 24 April 2006, UTM 16 304602E 4888113N, KJT (exuviae of 14 reared *E. spinigera*); Meilke Lake, 12 May 2007, UTM 16 315894E 4877729N, KJT (exuviae of 5 reared *E. spinigera*).

## RESULTS

Combining the data on total number of palpal + premental setae (= total setae) for the two species resulted in a bimodal curve (Fig. 1). Overlap occurred from 34 through 37 total setae, although few individuals were represented in this range. Approximately 95% of the *E. cynosura* had 35 or fewer total setae, whereas 96% of the *E. spinigera* had 36 or more total setae (Table 1). For *E. cynosura*, total setae counts did not differ among five lakes having the largest sample sizes ($H = 2.616$, df = 4, $P = 0.624$), and total setae number was not correlated with total length of exuviae ($r = -0.0673$, N = 65, $P = 0.594$). However, for *E. spinigera*, total setae counts differed significantly among four lakes having the largest sample sizes ($H = 9.427$, df = 3, $P = 0.024$), and total setae number was positively correlated with total length of exuviae ($r = 0.381$, N = 54, $P = 0.005$).

Numbers of premental setae also resulted in a bimodal distribution, but substantial overlap occurred from 20 to 23 setae, with most overlap at 22 setae (Table 1). For *E. cynosura*, 86% of exuviae had 21 or fewer premental setae, with most exuviae having 19 to 21 setae. For *E. spinigera*, 96% of exuviae had 22 or more premental setae, with most exuviae having 22 to 25 setae. Exuviae of both species often had unequal numbers of setae on the two sides of their prementum; this occurred on 51% of *E. cynosura* and 56% of *E. spinigera*.

Substantial overlap in numbers of palpal setae between species occurred from 13 through 15 setae and the single highest percentage of both species had 14 setae (Table 1). Fifty nine percent of *E. cynosura* had 13 or fewer palpal setae, while only 4% of *E. spinigera* had that number. Twenty six percent of *E. spinigera* had 15 or more palpal setae, while only 3% of *E. cynosura* had that number. Exuviae of both species sometimes had unequal numbers of setae on their two labial palps; this occurred on 34% of *E. cynosura* and 22% of *E. spinigera*.

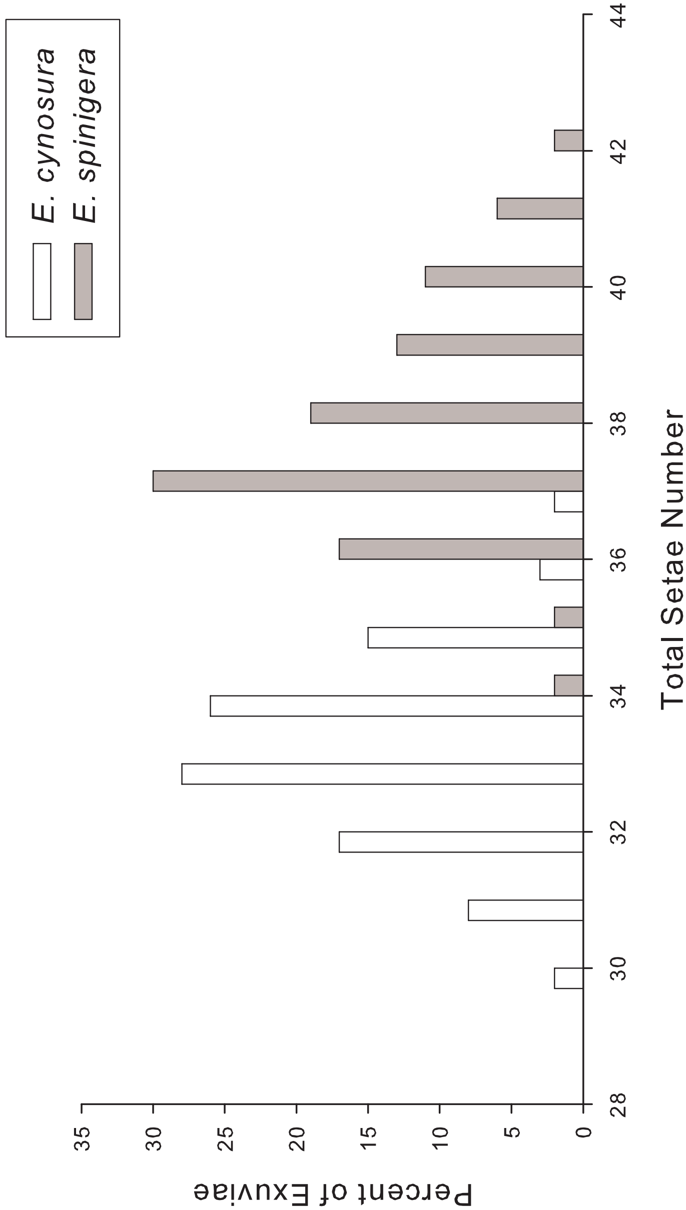


Figure 1. Numbers of total raptorial setae (left + right palpals plus left + right prementals) possessed by reared exuviae of *Epitheca cynosura* and *E. spinigera* from Wisconsin.

Table 1. Frequency of exuviae with number of raptorial setae (left + right palpals, left + right prementals, and total) in reared *Epitheca cynosura* (n = 65) and *E. spinigera* (n = 54) from Wisconsin.

| **Palpals** | **12** | **13** | **14** | **15** | **15** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *E. cynosura* | 18 | 20 | 25 | 2 | 0 | | | | | |
| *E. spinigera* | 0 | 2 | 38 | 9 | 5 | | | | | |
| **Prementals** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **26** |
| *E. cynosura* | 1 | 3 | 14 | 23 | 15 | 8 | 1 | 0 | 0 | 0 |
| *E. spinigera* | 0 | 0 | 0 | 1 | 1 | 10 | 17 | 12 | 8 | 5 |
| **Total** | **≤31** | **32** | **33** | **34** | **35** | **36** | **37** | **38** | **39** | **≥40** |
| *E. cynosura* | 6 | 11 | 18 | 17 | 10 | 2 | 1 | 0 | 0 | 0 |
| *E. spinigera* | 0 | 0 | 0 | 1 | 1 | 9 | 16 | 10 | 7 | 10 |

Exuviae of *E. spinigera* averaged 22.9 mm in total length, while exuviae of *E. cynosura* averaged 21.9 mm in total length, but the overlap between species was substantial. Differences in mean exuviae length among lakes were evident for both species that could have been attributable to lake effects, inter-annual effects, or both. For *E. cynosura* total exuvia length was significantly different among four lakes sampled in 2005 ($H = 13.195$, df = 3, $P = 0.004$). Significant differences among lakes sampled in 2007 were found also for *E. spinigera* ($F = 13.221$, df = 3, $P < 0.001$). Our data allowed for only one direct test of an inter-annual effect on total exuviae length, at a pond at Memory Lake Campground in Burnett County, where *E. spinigera* were sampled in 2005, 2006, and 2007. Here we did not find evidence of an effect attributable to year ($H = 1.566$, df = 2, $P = 0.457$).

Female exuviae of *E. spinigera* had longer cerci than did female exuviae of *E. cynosura*. This was most clearly seen in the ratio obtained by dividing the mean length of both cerci by the length of the epiproct. Despite some overlap, the cerci ÷ epiproct ratio was < 0.75 for all female *E. cynosura*, and ≥ 0.75 for 90% of female *E. spinigera* (Table 2). We did not find a difference between species in the cerci ÷ epiproct ratio for male exuviae ($T = 821.5$, N = 65, $P = 0.188$).

The position of the ante-apical tubercles on the epiproct of male exuviae, expressed as the ratio of the distance from the base of the epiproct to the distal margin of the tubercle ÷ the total length of the epiproct, differed between species. The tubercles of *E. cynosura* were located more distally on the epiproct than those of *E. spinigera* (Table 2). However, the difference between species was slight and there was some overlap. Nearly all (98%) *E. cynosura* had a tubercle distance from base ÷ epiproct length ratio ≥ 0.66, whereas 73% of *E. spinigera* had values < 0.66.

The shape of the dorsal hook on S8, with respect to the direction in which the tip was pointed in strict lateral view, sometimes differed between species (Fig. 2). Often (55% of *E. cynosura*; 36% of *E. spinigera*), the tip of the dorsal hook on S8 pointed straight rearward in line with the long axis of the body. In these cases, the character would have had no diagnostic value. However, among the remainder, the tip of the hook pointed either slightly downward (ventrally – Fig. 2a) or slightly upward (dorsally – Fig. 2b) relative to a long-axis body line.

Table 2. Mean character ratios of reared exuviae of *Epitheca cynosura* and *E. spinigera* from Wisconsin (SE in parentheses).

| **Species** | **♀ Cerci/ epiproct** | **♂ Base to tubercle/ epiproct** | **Spine of 8/ margin of 8** | **Spine of 9 W at base/L** | **Abdomen W at S6/L** |
|---|---|---|---|---|---|
| *E. cynosura* | 0.656 (0.00746) | 0.708 (0.00381) | 0.181 (0.00253) | 0.298 (0.00458) | 0.685 (0.00275) |
| *E. spinigera* | 0.783 (0.00701) | 0.645 (0.00623) | 0.163 (0.00211) | 0.301 (0.00533) | 0.692 (0.00416) |

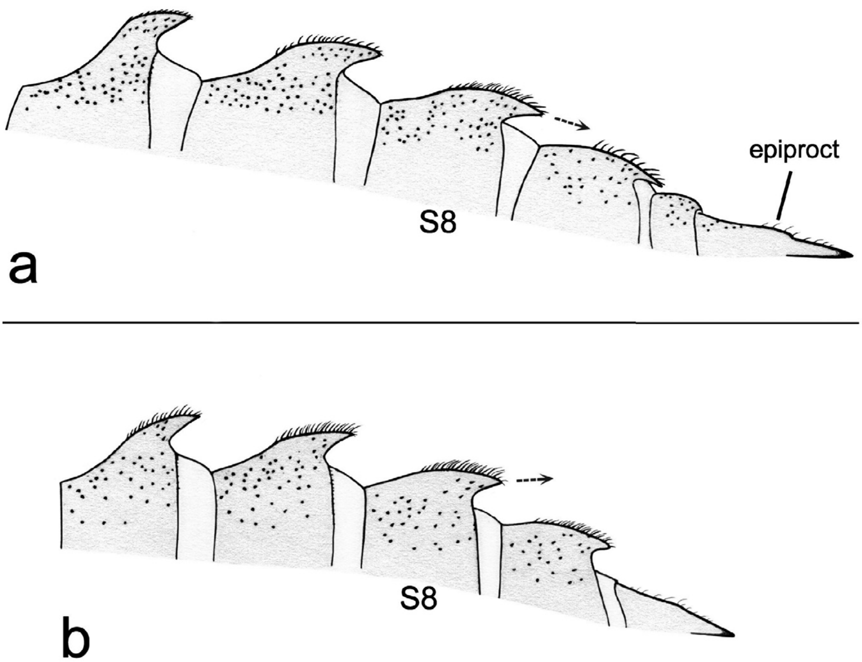


Figure 2. Dorsum of abdomen in lateral view of exuviae of *Epitheca spinigera* (a) and *E. cynosura* (b); arrows indicate direction of S8 hook relative to long axis of body (represented by horizontal line).

All cases in which the tip pointed slightly upward were *E. cynosura*, and 93% of cases in which the hook pointed slightly downward were *E. spinigera*.

The lateral spines on S8, relative to the length of the lateral edge of the segment including the spine, averaged longer on *E. cynosura*, but the mean difference in this ratio between species was slight (Table 2) and there was substantial overlap. The slenderness of the left lateral spine on S9, expressed as the ratio of width at the base ÷ length, did not differ between species (Table 2). The shape of the left lateral spine on S9 (straight *vs*. slightly incurved) also did not differ between species ($\chi^2 = 1.002$, df = 1, $P = 0.317$).

The shape of the abdomen (ratio of the maximum width at S6 ÷ total abdomen length) did not differ between exuviae of the two species ($T = 3028.0$, $P = 0.229$; Table 2). The maximum width of the abdomen at S6 was greater for *E. spinigera* (avg. = 8.6 mm) than for *E. cynosura* (avg. = 8.1 mm); however, this difference was attributable to the larger mean size of *E. spinigera*, not to a difference in shape, and there was substantial overlap in the character.

## DISCUSSION

Raptorial setae counts reliably distinguished between reared exuviae of *E. cynosura* and *E. spinigera*, but only if all premental setae, or preferably, total setae (all premental + all palpal setae) were counted. An optimal couplet using total setae counts (*E. cynosura* ≤ 35; *E. spinigera* ≥ 36) was most effective, and would have correctly determined 96% of the reared exuviae in our sample, with no substantial overlap at any single number of total setae. An optimal couplet using just premental setae counts (*E. cynosura* ≤ 21; *E. spinigera* ≥ 22) would have correctly determined 91% of the reared exuviae in our sample, with substantial overlap occurring only at 22 setae. The increase in efficacy gained by use of total setae counts over just premental setae counts is worthwhile and easily attained because palpal setae are easy to count, and the labial palps are exposed and readily visible when the setae on the prementum are counted. Palpal setae counts alone were unreliable for distinguishing between these species because there was considerable overlap at 14 setae, and the most reasonable diagnostic couplet using palpal setae (*E. cynosura* ≤ 13; *E. spinigera* ≥ 14) would erroneously determine 42% of *E. cynosura* as *E. spinigera*.

When setae counts are used, we urge that setae on both sides of the prementum and on both labial palps be counted because we frequently found unequal numbers of these setae on the two sides of exuviae of both species. Counting setae on both sides of both structures provides results that are less ambiguous and less confusing than employing counts on only one side, as has been the current practice (Walker and Corbet 1975, Bright and O'Brien 1999, Needham et al. 2000). For example, premental setae counts were used in these keys as either the sole character, or the primary character, to separate *E. spinigera* (11 or more setae on one side) from *E. cynosura*, or the group containing *E. cynosura* (10 or fewer setae on one side). Based on our data, this character would erroneously determine 14% of *E. cynosura* that have at least 11 setae on each side of the prementum, and would leave ambiguous another 25% of *E. cynosura* that have 11 setae on one side of the prementum. Thus, the character would correctly and unambiguously determine only 62% of *E. cynosura*. Adding palpal setae counts to the couplet as a secondary character (Bright and O'Brien 1999, Needham et al. 2000) did not reduce the confusion because a substantial proportion of both species have unequal numbers of palpal setae on their two sides.

We do not generally support use of total exuviae length as a diagnostic character to distinguish these species even though *E. spinigera* is often somewhat larger. Difficulties could occur because the area of overlap in length is extensive, and some lakes have small *E. spinigera*. Kormondy (1959) noted a north-south cline of last-instar size in Michigan, with smaller individuals in the southern part of the state. Exuviae ≥ 24 mm in total length in Wisconsin are at least 12 times more likely to be *E. spinigera* than *E. cynosura*. However, only 38% of *E. spinigera* in our sample attained that size. If total setae counts of a last-instar nymph or exuviae are in the center of the region of overlap between species (35 or 36 setae), then a total length ≥ 24 mm would strongly indicate *E. spinigera*. The findings that total lengths of exuviae of both species varied among lakes, and that total lengths of *E. spinigera* were positively correlated with total setae counts, suggest that separating these species using setae counts will be most challenging with small *E. spinigera* that sometimes occur in certain lakes. Our inability to demonstrate an inter-annual effect on total exuviae length for *E. spinigera* over three years at just one site does not necessarily mean that this character does not vary among years in either species at other sites.

The finding that the cerci ÷ epiproct ratio differed significantly between female exuviae of these species was not unexpected because adult female *E. spinigera* have considerably longer cerci than do adult female *E. cynosura*. This ratio could have limited utility as an ancillary diagnostic character to separate female exuviae and last instar nymphs of these species (cerci at least 3/4 the length of the epiproct = *E. spinigera*; cerci less than 3/4 the length of the epiproct = *E. cynosura*). Although there is relatively little overlap between species, the cerci ÷ epiproct ratio has drawbacks that would reduce its diagnostic value in most circumstances including: 1) most specimens of both species are quite close to the 0.75 ratio cutoff value, requiring precise measurements to be made, 2) the two cerci on a single specimen may differ slightly in length, requiring that both be measured and averaged, which takes additional time, 3) the character can be applied to specimens of only one gender, and 4) reliability in excess of 95% can be achieved using the simpler character of total setae counts alone. However, in cases where last-instar nymphs and exuviae have total setae counts of 35 or 36, and total lengths that are < 24 mm, calculating the cerci ÷ epiproct ratios of females could aid in making determinations.

The finding that the position of the ante-apical tubercles on the epiprocts of male exuviae differs somewhat between species also has limited diagnostic value, because as with the previous character, the difference was slight (thus requiring exacting measurements), there was some overlap, the character can be applied to only part of the population, and a better diagnostic character exists. As with the cerci ÷ epiproct ratios of females, the tubercles ÷ epiproct ratios of males (< 0.66 = *E. spinigera*; ≥ 0.66 = *E. cynosura*) could be helpful to weigh into the identification process in cases where last-instar male nymphs and exuviae have total setae counts of 35 or 36, and total lengths < 24 mm.

We hesitate to recommend use of dorsal hook and lateral spine characters to separate these species, even though some significant differences were found, because substantial variation occurred in these characters that could have been environmentally induced. The direction in which the tip of the dorsal hook on S8 points could cautiously be used as an ancillary character to reinforce determinations made using other characters. In the approximately 54% of the exuviae of the two species in our sample in which the tip of this hook did not point straight rearward in line with the long axis of the body, an upward pointing tip strongly indicated *E. cynosura* and a downward pointing tip suggested *E. spinigera*, though somewhat less strongly. Although the lateral spine on S8 averaged significantly longer relative to the length of the segment margin on *E. cynosura*, the difference between the species was slight and there was considerable overlap, which reduced the diagnostic value of the character. Our assessments of the shape and dimensions of the left lateral spine on S9 led us to the same conclusion reached by Walker (1913) and Kormondy (1959), that there are no useful diagnostic attributes associated with this spine.

Contrary to our expectations, the shape of the abdomen was highly variable for each species, and it did not differ between species. Part of the reason for this variation could have been associated with the difficulty, because of the various contorted shapes of exuviae, of depressing the sclerite on the venter of segment 6 in a consistent way to determine maximum width, and of obtaining a consistent value for total abdomen length.

## RECOMMENDATIONS

Use of total setae counts (*E. cynosura* ≤ 35; *E. spinigera* ≥ 36) is the most effective way to separate exuviae and last-instar nymphs of these species. These counts correctly determined 96% of the reared exuviae in our sample, with no substantial overlap at any single number of total setae. For specimens in the center of the area of slight overlap (35 or 36 total setae), several ancillary characters can be used to reinforce determinations including: total length (if ≥ 24 mm – *E. spinigera*), cerci ÷ epiproct ratio for females (*E. cynosura* < 0.75; *E. spinigera* ≥ 0.75), distance to ante-apical tubercle ÷ epiproct for males (*E. spinigera* < 0.66; *E. cynosura* ≥ 0.66), and the direction the tip of the dorsal hook on segment 8 projects in lateral view (slightly upward – *E. cynosura*; slightly downward – *E. spinigera*). These recommendations are intended for application only with last-instar nymphs and exuviae and only within the region we sampled (Wisconsin). Extrapolation of these recommendations with younger nymphs or outside this region should be undertaken cautiously.

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