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Morphological Differentiation and Adaptation of the Larvae of *Rana berlandieri* and *Rana sphenocephala* (*Rana pipiens* complex) in Sympatry

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Syntopic larvae of *Rana berlandieri* and *Rana sphenocephala*, both of the *Rana pipiens* complex, were analyzed for morphological differentiation using multivariate techniques. Field-collected and laboratory-reared larvae of both species were compared for 12 morphometric characters.

***R. berlandieri* larvae differ from *R. sphenocephala* larvae in having a more fusiform body shape, greater tail musculature development, a longer tail, relatively narrow dorsal and ventral tail fins, a shorter gastrointestinal tract length, fewer oral papillae on the lower labium, and a smaller ratio of interocular distance/internareal distance. The number of oral papillae, however, is phenotypically plastic and probably varies with diet or mode of feeding. Pigmentation differences include tail blotching in *R. berlandieri* vs relatively uniform melanophore distribution in the tail fin of *R. sphenocephala*. Most of the morphological differentiation can be explained on the basis of larval habitat and dietary differences between these two species. *R. berlandieri* larvae are stream-adapted and probably more benthivorous than are the pond-adapted, planktivorous *R. sphenocephala* larvae.**

THE obscure morphological differentiation of adults among the various species of the *Rana pipiens* complex once resulted in taxonomic instability within this group (Pace, 1974). Only recently have most of the United States species of the *R. pipiens* complex been adequately defined; most of the Central American species are yet to be described or allocated to a specific taxon. Tadpoles of even the best-known species within this group often cannot be identified with published information (Wassersug, 1976), which has led to further taxonomic confusion (Tucker, 1976). To date, the only leopard frog larvae that have been characterized morphologically in the literature are *R. blairi* and *R. pipiens* (Korky, 1978).

Hillis (1981) reported breeding habitat distinctions between *Rana berlandieri* and *Rana sphenocephala* (=*R. utricularia* sensu Pace, 1974) in central Texas. *R. berlandieri* naturally breeds primarily in streams, whereas *R. sphenocephala* typically breeds in non-flowing water. Therefore, the larvae of these two species should show differential morphological adaptations to their respective habitats. In areas where syntopic breeding does occur, *R. berlandieri* usually breeds in the fall or early winter and *R. sphenocephala* breeds in late winter or early spring. Because of temporal differences of food-type availability in the larval habitat there may be differences in the diets of the larvae of these two species; these differences could be reflected in the morphological structures associated with feeding in the two species.

This study was undertaken to find a means of distinguishing the larvae of *R. berlandieri* from the larvae of *R. sphenocephala* and to test the above predictions regarding adaptation to habitat and diet in these two species.

METHODS

Four samples of 25 tadpoles each were used in the analyses. One sample of each species was collected from a floodplain lake of the Brazos River at Waco, McLennan County, Texas and preserved in 5% formalin. Two samples, one of each species, were reared from eggs taken from egg masses of identified adults at the above locality. The laboratory groups were reared under similar conditions and fed an agar/gelatin/rabbit food diet as described by Nace et al. (1974). Water temperature was maintained at 21 ± 1 C.

Specimens of stages 28–40 (Gosner, 1960) were measured to the nearest 0.1 mm with dial calipers after Altig's (1970) methods (Table 1). Tail musculature height and tail musculature thickness were measured just posterior to the body. Gastrointestinal tracts were removed

TABLE 1. COMPARISON OF MEANS ± 1 SD AND RANGES OF 12 MORPHOLOGICAL CHARACTERS OF FIELD-COLLECTED *Rana berlandieri* AND *R. sphenocephala* LARVAE AND THE FACTOR LOADINGS OR PRINCIPAL COMPONENTS I AND II.

Character (in mm except #10 and #12)	*R. sphenocephala* N = 25	*R. berlandieri* N = 25	Factor loadings	
	$\bar{x}$ ± 1 SD (range)	$\bar{x}$ ± 1 SD (range)	PC I	PC II
1. Body length	24.7 ± 1.7 (21.0–27.6)	27.2 ± 3.1 (19.7–31.5)	0.89	−0.30
2. Tail length	41.5 ± 5.2 (28.5–49.3)	51.7 ± 8.1 (34.4–69.6)	0.81	−0.43
3. Tail height	18.6 ± 1.9 (13.2–21.4)	17.9 ± 2.9 (12.9–27.8)	0.80	0.30
4. Tail musculature height	8.12 ± 1.0 (6.6–10.1)	9.86 ± 2.9 (6.2–19.0)	0.69	−0.41
5. Tail musculature thickness	5.44 ± 0.78 (3.6–6.9)	7.68 ± 1.3 (5.0–9.5)	0.74	−0.61
6. Ventral fin height	5.56 ± 0.83 (3.8–7.1)	4.83 ± 0.78 (3.5–6.5)	0.40	0.73
7. Dorsal fin height	6.84 ± 0.90 (4.8–8.7)	5.96 ± 0.80 (3.8–7.2)	0.58	0.71
8. Interocular distance	6.76 ± 0.94 (4.6–8.9)	6.14 ± 0.94 (4.2–8.3)	0.71	0.50
9. Internareal distance	2.97 ± 0.39 (1.8–3.6)	3.14 ± 0.37 (2.5–3.7)	0.63	0.11
10. Oral papillae	46.2 ± 8.1 (31–62)	32.0 ± 7.3 (20–54)	−0.17	0.70
11. Intestine length	472 ± 50.5 (380–560)	388 ± 71.1 (240–514)	0.43	0.71
12. Mass (g)	4.10 ± 0.85 (2.24–5.30)	5.31 ± 1.79 (1.64–7.74)	0.94	−0.24

from the field-collected specimens and were measured to the nearest millimeter. Body mass was measured to 0.01 g with a Mettler balance. Number of marginal oral papillae on the lower labium (a character herein called "oral papillae") was counted with the aid of a dissecting microscope.

The intestines of the field-collected specimens were emptied and the contents were examined microscopically. All algae were identified to genus.

In order to differentiate between field-collected specimens of the two species, a principal components analysis was performed by use of program BMDP4M in the statistical package described by Dixon (1975). The data matrix included all characters listed in Table 1. The program extracted 12 orthogonal principal components by use of a correlation matrix among the variables. Each is a weighted linear function of the original variables which accounts for the maximum amount of variance remaining after all variance accounted for by preceding components is removed. Morrison (1967) and Schnell (1970) further describe the technique.

Environmental versus genetic effects on the morphology of the tadpoles were assessed in two stepwise discriminant function analyses (program BMDP7M of the statistical package described by Dixon, 1975). All characters in Table 1 except intestine length were used in these evaluations. In the first analysis, the discriminant function was computed for field-collected specimens of the two species (after identification based upon known breeding dates of the two species at the collection site and subsequent transformation of non-preserved specimens from the same collections). Discriminant scores of this function were then computed for the laboratory-reared specimens. To determine which characters best separate the two species under identical rearing conditions, a second analysis was performed in which the discriminant function was computed for the laboratory-reared tadpoles; scores for this function were then computed for the field-collected tadpoles.

RESULTS

The first factor (PC I) extracted by the principal components analysis was size-related. It accounted for 46.7% of the variance within the lumped field-collected sample. The second factor (PC II) accounted for 27.2% of the variance and produced two nearly distinct subgroups corresponding to *R. berlandieri* and *R. sphenocephala* when plotted against PC I (Fig. 1). The factor loadings of PC I and PC II are listed in Table 1.

The discriminant function analysis using only the field-collected specimens as a reference completely separated the two field-collected samples (one of each species) on the basis of oral papillae, tail length, dorsal fin height, interocular distance, internareal distance and tail musculature thickness (Fig. 2A). Scores of the

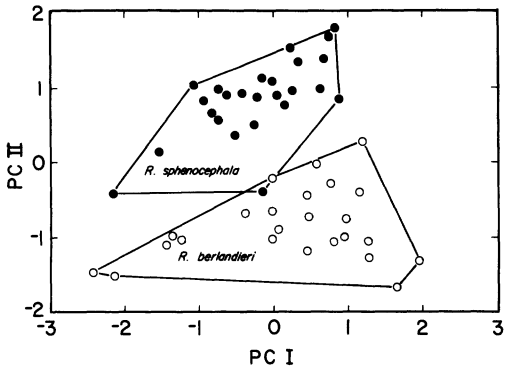

Fig. 1. Comparison of the scores of field-collected *Rana berlandieri* and *R. sphenocephala* larvae on principal components I and II. See Table 1 for the factor loadings of PC I and PC II.

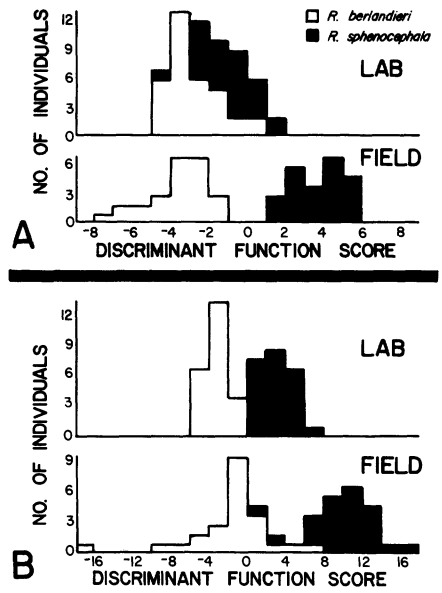

Fig. 2. Stepwise discriminant function separation of *Rana berlandieri* and *R. sphenocephala* larvae. A) Discriminant function defined on the basis of field-collected specimens. B) Discriminant function described on the basis of laboratory-reared specimens. See text for characters used in defining both discriminant functions.

laboratory-reared samples of the two species widely overlapped, however. This overlap was due primarily to retention of oral papillae as the most important character used in distinguishing the field-collected samples, whereas laboratory-reared specimens of *R. sphenocephala* converged toward the *R. berlandieri* state for this character (Fig. 3). In the second discriminant function analysis, when laboratory-reared samples were used to define the discriminant function, laboratory-reared samples were completely separated and the field-collected samples exhibited about 12% overlap (Fig. 2B). Scores of the field-collected *R. berlandieri* tended toward the scores of laboratory-reared *R. sphenocephala.* In this analysis the oral papillae count was not selected by the program for discrimination: instead tail length, interocular distance, mass, tail muscular thickness, dorsal fin height, and body length were used.

The plot of gastrointestinal tract length versus body mass is shown in Fig. 4. Field-collected specimens of *R. sphenocephala* larvae had significantly longer gastrointestinal tract lengths than *R. berlandieri* larvae ($F = 2456$, $P \ll .001$). The least squares regression lines indicate an increase of 35.4 mm of gastrointestinal tract per gram body mass in *R. sphenocephala* and an increase of 28.2 mm of gastrointestinal tract per gram body mass in *R. berlandieri* ($F = 1.934$, not significantly different).

The inspection of the intestinal contents of the field-collected specimens revealed a significant difference in the algae present in each of the two species. The intestines of *R. berlandieri* contained 12 genera of diatoms; these diatoms accounted for the bulk of the identifiable intestinal contents of this species, although 6 genera of green algae and 5 genera of blue-green algae were present in much smaller numbers. Diatoms were much less common in the *R. sphenocephala* intestines; 7 genera were present, only one (the planktonic *Dinobryon*) in significant numbers. Planktonic green algae (especially *Pleurotaenium*) made up the bulk of the contents of the *R. sphenocephala* intestines; blue-green algae were few and of about the same relative abundance as in *R. berlandieri.* The intestines of the *R. berlandieri* tadpoles also contained more inorganic particles than did the intestines of the *R. sphenocephala* tadpoles.

Pigmentation differences were also noted between the two species. *Rana berlandieri* tadpoles have prominent concentrations of melanophores on the tail fins, producing a blotched pattern. *Rana sphenocephala* tadpoles have a more uniform distribution of melanophores on the tail fins (Fig. 5). While this character varies greatly depending upon water quality, temper-

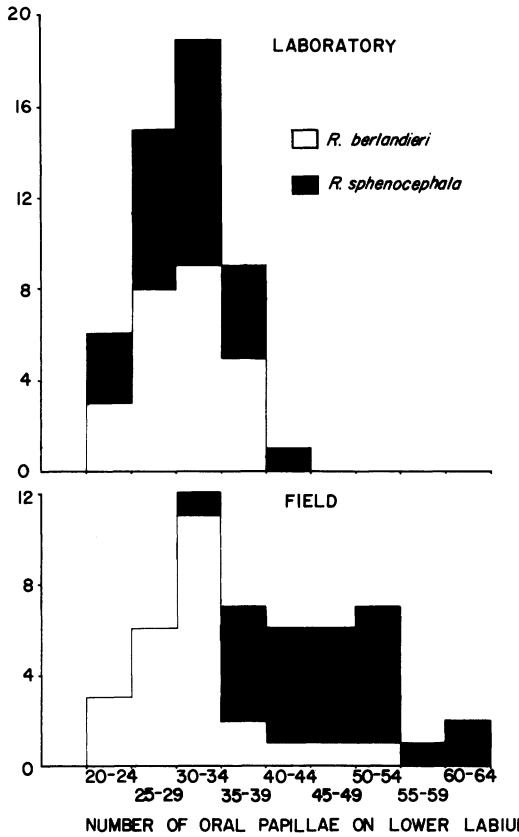

Fig. 3. Comparison of number of oral papillae on lower labium of the larvae of *Rana berlandieri* and *R. sphenocephala* in laboratory-reared and field-collected samples.

ature, and light conditions, it is often adequate for species identification.

Discussion

Field-collected *R. sphenocephala* larvae are characterized by having relatively shorter, less muscular tails with higher tail fins, more oral papillae, and longer gastrointestinal tracts than field-collected, syntopic *R. berlandieri* (Table 1; Figs. 4, 5). The ratio of tail length/dorsal fin height completely separates the two groups and can be used for identification purposes ($\bar{x} \pm 1$ SD [range] for *R. berlandieri* = 8.71 ± 0.90 [7.2–10.6]; for *R. sphenocephala* = 6.10 ± 0.61 [4.9–7.0]); also useful for identification of the two species are tail pigmentation and tail musculature differences.

The morphological convergence of the laboratory-reared specimens compared to the field-collected specimens (Fig. 2A) has several implications. The most obvious convergence is in the number of oral papillae (Fig. 3). Lack of differentiation in number of oral papillae in laboratory-reared specimens of the two species may result from phenotypic plasticity and the uniformity of the laboratory environment. No function has been described for oral papillae (Altig, 1970), but a feeding (sensory) function is probable, and laboratory tadpoles were fed identical diets on identical substrates. Besides food and substrate, other environmental differences (i.e., developmental temperature) which were not present in the laboratory populations may have led to the differentiation in oral papillae (and possibly other characters) of the field-collected larvae.

The intestinal analysis of the two species supports the hypothesis of differing feeding habits of *R. berlandieri* and *R. sphenocephala* tadpoles. The *R. berlandieri* tadpoles contained many benthic diatoms, as well as more inorganic matter than did the *R. sphenocephala* tadpoles. The *R. sphenocephala* tadpoles contained mostly planktonic green algae. It is highly unlikely that *R. berlandieri* tadpoles digest diatoms to any significant degree due to the silicious encasings of these algae. Instead, *R. berlandieri* may ingest bottom detritus indiscriminately (thus ingesting diatoms incidentally), digesting bacteria, protozoans, small metazoans, and pieces of carrion. Since these food items would be digested much more quickly than silicious diatoms, the diatoms are greatly in evidence in the intestinal tracts of *R. berlandieri* tadpoles. The *R. sphenocephala* tadpoles, however, appear to feed mainly by "filtering" the water above the bottom, thus ingesting (and presumably digesting) more planktonic green algae than do *R. berlandieri* larvae. However, this may not be a species-specific trait; Hendricks (1973) suggested that leopard frog larvae may shift feeding modes depending upon available food supply.

Other indirect evidence supports the hypothesis of differing feeding habits for *R. berlandieri* and *R. sphenocephala* larvae. *R. sphenocephala* in sympatry with *R. berlandieri* typically breed in late winter or early spring (Hillis, 1981); this is just before the period of greatest abundance of green algae (Smith, 1950). Therefore, planktonic green algae are readily available throughout the development of the *R. sphenocephala* tadpoles. However, since sympatric *R. berlandieri* typically breed in fall or early winter (Hil-

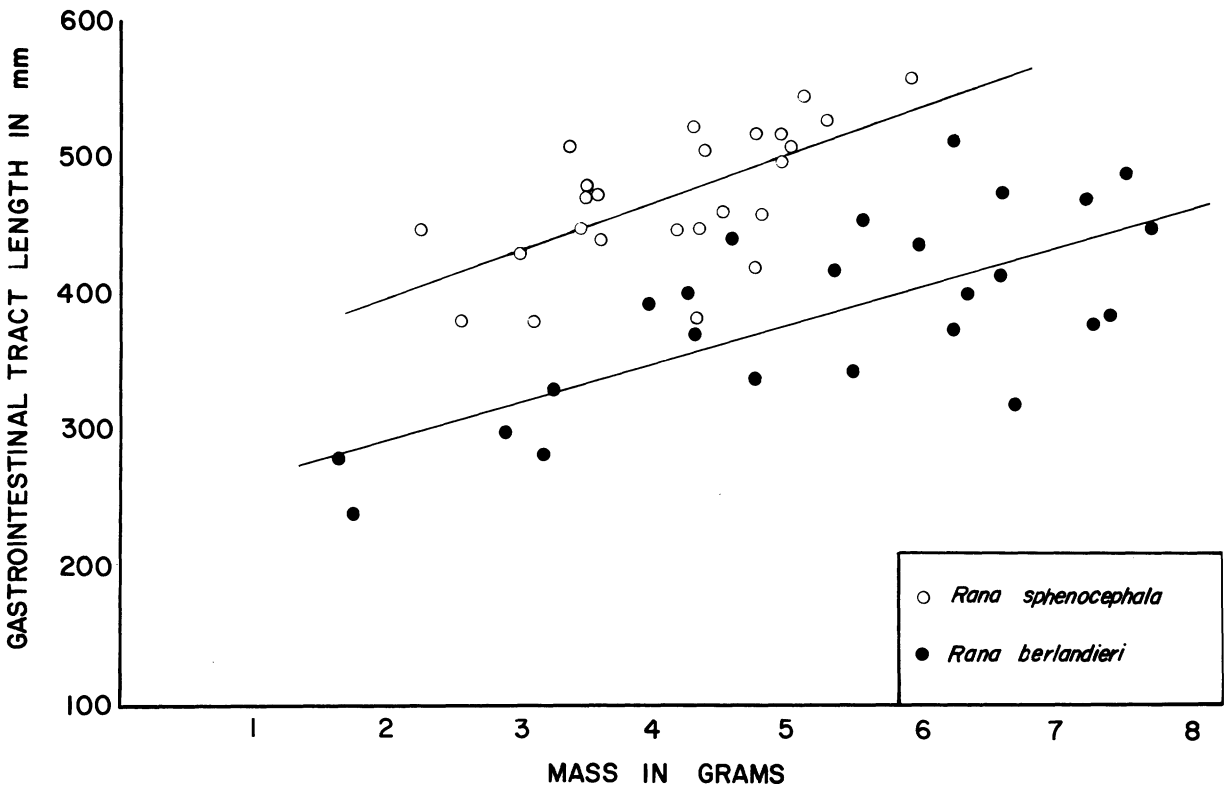

Fig. 4. Gastrointestinal tract lengths of field-collected *Rana berlandieri* and *R. sphenocephala* larvae as a function of body mass.

lis, 1981), digestable algae are much less available through the winter development of the tadpoles; this situation may necessitate benthivory. This is supported by the relatively longer gastrointestinal tract of *R. sphenocephala* larvae, indicative of a more herbivorous diet, compared to *R. berlandieri* larvae (Fig. 4). In addition, I have observed large *R. berlandieri* tadpoles in the laboratory consume smaller siblings, whereas I have not observed this phenomenon among *R. sphenocephala* tadpoles. Since *R. berlandieri* tadpoles are usually relatively large when *R. sphenocephala* tadpoles hatch, it is possible that *R. berlandieri* tadpoles eat *R. sphenocephala* tadpoles at sympatric localities.

Collins and Lewis (1979) reviewed the species of North American *Rana* which overwinter as tadpoles. *Rana berlandieri* should be added to this list. It would be of interest to check the other species in which larvae regularly overwinter for benthivorous or carnivorous feeding habits. This correlation of feeding habits with overwintering would only apply to areas where winter restricts algal growth.

Most of the remaining morphological differences between *R. berlandieri* and *R. sphenocephala* tadpoles are correlated with the typical larval habitat of each species. Thus *R. berlandieri* tadpoles, adapted for streams, are more fusiform and have more-muscular tails with lower tail fins than do *R. sphenocephala* tadpoles, which are adapted for non-flowing water. It is also possible that benthivorous feeding habits of *R. berlandieri* are an additional adaptation to stream-dwelling, since phytoplankton growth is likely to be less extensive in flowing water.

Although not noted by Korky (1978), it appears from his illustrations that *R. blairi* tadpoles have much more muscular tails than *R. pipiens* tadpoles. Korky did not measure either tail musculature thickness or height, so future workers interested in the differentiation of these two species should investigate these char-

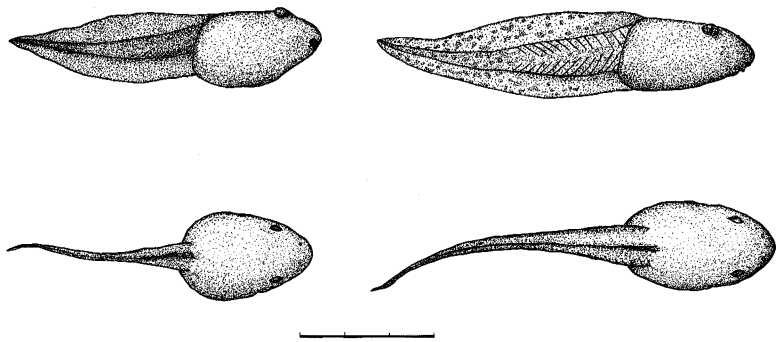

Fig. 5. Dorsal and lateral views of field-collected *Rana sphenocephala* (left) and *R. berlandieri* (right) larvae. Scale is marked in centimeters.

acters. It also should be noted that Korky (1978) adjusted all larvae in his samples to a mean stage of 34 (scale of Gosner, 1960), using his reported correlation between larval development stage and larval body length of r = 0.99. However, he did not take allometric growth of the tadpoles into account. Although correlation among the growth rates of the various body parts is high, in the present study none of the characters measured by Korky (1978) were correlated with body length as closely as r = 0.99. The correlations between Korky's morphometric characters and body length varied from r = 0.85 (tail height) to r = 0.33 (ventral fin height) among *R. sphenocephala,* and from r = 0.88 (tail length) to r = 0.46 (ventral fin height) among *R. berlandieri* (I did not measure Korky's "eye-nostril distance" in my samples). Body length did correlate well with developmental stage among my samples, although the correlations were lower than Korky's r = 0.99 (*R. berlandieri,* r = 0.94; *R. sphenocephala,* r = 0.81).

Despite the above-noted problems, it is likely that Korky's (1978) ratios of morphometric characters are generally representative of *R. blairi* and *R. pipiens* larvae. It is of interest, therefore, to compare them with *R. berlandieri* and *R. sphenocephala* larvae. Korky reported a significant difference between the internareal and interocular distances of *R. blairi* and *R. pipiens* larvae; *R. pipiens* larvae had larger measurements in both cases. Field-collected *R. berlandieri* and *R. sphenocephala* tadpoles both have larger interocular distances and smaller internareal distances than either *R. blairi* or *R. pipiens* (mean interocular distance/internareal distance is 1.31 for *R. blairi,* 1.34 for *R. pipiens,* 1.96 for *R. berlandieri,* and 2.28 for *R. sphenocephala*). Another interesting comparison among these four *Rana* larvae is of the tail length/body length ratios. *Rana blairi* and *R. pipiens* larvae both have relatively short tails in Korky's (1978) Nebraska samples (TL/BL respective means, 1.43 and 1.49) compared to field-collected *R. sphenocephala* (1.68) and especially *R. berlandieri* (1.90) in Texas.

Korky (1978) concluded that *R. blairi* and *R. pipiens* larvae are only slightly differentiated and stated that "constraints imposed by a reproductive strategy utilizing oviposition in an aquatic medium may be severe and result in little interspecific differentiation at this stage in the life cycle." This statement is contradicted by the differentiation of larvae seen among some other genera of anurans. Aquatic habitats are highly variable and correlated specific differentiation should be expected. Furthermore, no adaptational significance has been reported for any of the morphological differences of adults of the *R. pipiens* complex (Moore, 1975). Apparently, the larval habitats of *R. berlandieri* (chiefly streams) and *R. sphenocephala* (chiefly standing water) are more distinct than those of *R. blairi* and *R. pipiens,* since the former pair are morphologically quite distinct and show correspondingly different adaptations to larval habitat.

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