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Hemoparasites in Oregon Spotted Frogs (*Rana pretiosa*) from Central Oregon, USA

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ABSTRACT: Between 2001 and 2003, we screened blood smears of 156 Oregon spotted frogs (*Rana pretiosa*) from three populations in central Oregon for blood parasites. A *Lankesterella* sp. and a *Trypanosoma* sp. were detected in 31% and 35% of the frogs, respectively. Parasite loads were generally light, with *Lankesterella* sporozoites in 1–2% of erythrocytes, and extracellular trypanosomes were seen at rates of about one parasite per 200 fields of view at 1000×. Little work has been published on hemoparasites of ranids in the western USA in the past 30 yr. Because of the recent taxonomic division of the *Rana pretiosa* complex, this may be the first published report of blood parasites for *R. pretiosa* sensu stricto. Both parasites reported here differed in morphologic features and morphometric comparisons from previous descriptions of anuran hemoparasites. Much work remains to sort out the taxonomy of hemoparasites among western USA ranids and to determine the ecological significance of these parasites; both tasks are important steps in understanding and managing these, and related, sensitive and threatened species.

Key words: Hemoparasites, *Lankesterella*, Oregon spotted frog, *Rana pretiosa*, *Trypanosoma*.

Amphibians host a variety of blood parasites, but there are relatively few published surveys of the hemoparasites of anurans of the northwestern USA (Lehmann, 1959a; Clark et al., 1969). Taxonomic revisions of western ranids (Green, 1986; Green et al., 1997), and the apparent host specificity of anuran hemoparasites (Martin and Desser, 1991), suggest a need for fresh investigations of hemoparasites in this group of frogs.

Lehmann (1959b, c, d) reported *Karyolysus sonomae*, *Haemogregarina boyli*, and *Trypanosoma boyli* from *Rana boylii*. A probable new trypanosome species, *Haemogregarina aurora*, was reported from *Rana aurora aurora* (Lehmann, 1960). Less information is available for the *R. pretiosa* complex in the Pacific Northwest. Lehmann (1959a) did not detect blood parasites in *R. pretiosa* from the Willamette Valley. Clark et al. (1969) reported a *Lankesterella* sp. and a *Trypanosoma* sp. in *R. pretiosa*.

The spotted frog complex was recently divided into *R. pretiosa* sensu stricto and *R. luteiventris* (Green et al., 1997). Although Clark et al. (1969) did not identify specific collection sites, much of that survey was done in Idaho, eastern Washington, and eastern Oregon, and the reported results probably relate to *R. luteiventris* rather than to *R. pretiosa*. Both *R. pretiosa* and *R. luteiventris* are candidate species for federal listing, and *R. pretiosa* is considered a "sensitive and critical" species throughout its range in Oregon (Oregon Natural Heritage Program [ONHP], 2001); many populations currently are small and isolated. Understanding the role of parasites in the ecology of threatened or endangered species is important for the long-term management of these species. Threatened and endangered species and small in-bred populations may exhibit reduced parasite species richness (Altizer et al., 2007) or reduced disease resistance, resulting in higher parasite burdens (Whiteman et al., 2006). This study of blood parasites of *R. pretiosa* contributes to the overall understanding of the ecology of this species.

From December 2001 to December 2003, we opportunistically made blood smears from adult Oregon spotted frogs

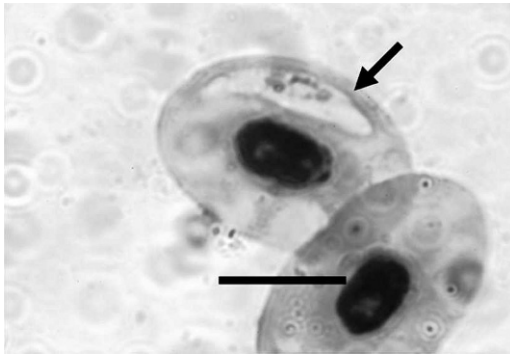

FIGURE 1. Intracellular *Lankesterella* sp. from *Rana pretiosa* within frog erythrocyte. Arrow = sporozoite in frog erythrocyte. Bar=10 μ.

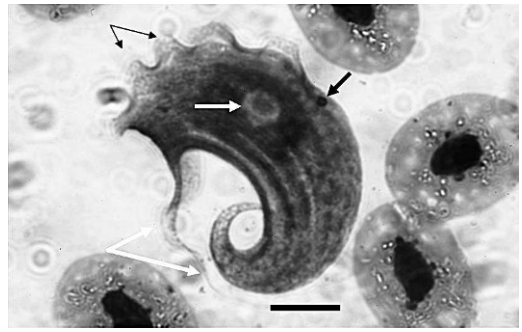

FIGURE 2. Trypomastigote of *Trypanosoma* sp. from *Rana pretiosa.* Single white arrow = nucleus; single black arrow = kinetoplast; paired black arrows = undulating membrane; paired white arrows = free flagellum. Bar=10 μ.

(*R. pretiosa*) during mark-and-recapture projects at three sites in Deschutes County, Oregon (Sunriver, NAD 27, UTM Zone 10, 624632 E, 4860501 N; Crosswater, 624358 E, 4856498 N; and Dilman Meadow, 607464 E, 4839143 N). Animals were toe-clipped and blood was applied directly to a glass microscope slide; a thin film was made using the trailing edge of a second glass slide. All frogs appeared normal and healthy and were released at the site of capture.

Slides were air dried, fixed in absolute methanol for 1 min, then air dried and stored at room temperature. Slides were immersed in freshly prepared Giemsa stain (LabChem Inc., Pittsburg, Pennsylvania, USA), diluted 1:30 with well water (pH 7) for 50 min at room temperature, rinsed under gently running well water, and air dried prior to examination. We initially examined slides at 100× (total magnification) and made final examination with oil immersion at 1000×. For measurement, we selected only parasites with intact cellular membranes. All measurements were made with an ocular micrometer (Wards 24 V 0230, Kyowa, Kanagawa, Japan) and were calibrated using a stage micrometer (Wards 24 V 0250, Kyowa). Length measurements were performed as described by Diamond (1965), without the use of a camera lucida. We measured the width of our *Lankesterella* at the widest point, while width of our *Trypanosoma* was measured at the nucleus. We determined the level of *Lankesterella* infection by counting the number of infected erythrocytes in 100 erythrocytes from successive fields of view. Ten total replicates were counted and averaged.

We found a *Lankesterella* sp. (Lankesterellidae) (Fig. 1), a hemococcidium, in 48 of 156 (31%) smears and a *Trypanosoma* sp. (Trypanosomatidae) (Fig. 2) in 54 of 156 (35%) smears (Table 1). Fourteen smears (9%) contained both parasites.

Stages of *Lankesterella* sp. were generally found as intraerythrocytic sporozoites, although 14 smears also exhibited extracellular sporozoites. Intracellular sporozoites appeared lightly stained within parasitophorous vacuoles in erythrocytes, but did not displace the erythrocyte nucleus. The vacuole was "banana-shaped" with one end usually broader. The parasite within the vacuole generally appeared elongated and slightly flexed, with a light reddish-staining, centrally located nucleus. There was often a bulge on the concave side of the vacuole opposite the nucleus. Extracellular sporozoites were elongate in form, stained lightly, and often showed greater flexion than the intracellular form. Parasites were generally found in fewer than 1–2% of the erythrocytes of

TABLE 1. Prevalence of hemoparasites in three populations of *Rana pretiosa* in Oregon.

Site	*n*	*Lankesterella* sp.	*Trypanosoma* sp.	Both
Sunriver	96	43 (45%)	25 (26%)	12 (13%)
Crosswater	51	5 (10%)	26 (51%)	2 (4%)
Dilman	9	0	3 (33%)	0
Total	156	48 (31%)	54 (35%)	14 (9%)

infected frogs. The most heavily infected frog had parasites in approximately 10% of the erythrocytes. We found no sporozoites within leukocytes.

The mean length of 245 intracellular sporozoites was 15.2 μ (range 11–21, SD 1.5) and the mean width was 3.4 μ (range 1.5–5, SD 0.6) (Table 2).

The *Trypanosoma* sp., circulating in the blood plasma, were primarily seen in flexed positions with a well-defined undulating membrane and free flagellum of varying lengths and were morphologically variable (Table 3). Most trypanosomes had a large oval nucleus (mean dimensions 6.9×5.2 μ) and a large central purple-staining endosome. Generally, the long axis of the nucleus oriented parallel to the longitudinal axis of the trypanosome. The nucleus was located just anterior to the middle of the body. The kinetoplast was small (~1 μ in diameter), circular, reddish-staining, and situated posterior to the nucleus by about 6.5 μ, with a clear space extending anteriorly from the kinetoplast. Buffered formalin was not used to fix the smears; this may have contributed to length variability of the flagella (Lehmann, 1964). Myonemes appeared prominently in about 43% of the parasites. Approximately 25% of the trypanosomes exhibited the "cornucopia" form reported for *T. ranarum* (Diamond, 1965). Trypanosome infections were light: At 1000× magnification, numbers of parasites seen ranged from a low of one or two total parasites, found in 200 fields of view, to a high of approximately 10 per 100 fields of view.

Our finding of both *Lankesterella* and *Trypanosoma* species in Oregon spotted frogs is similar to the results of Clark et al. (1969). However, Clark et al. probably sampled *R. luteveintris,* the Columbia spotted frog. The *Lankesterella* sp. and *Trypanosoma* sp. described in this study differed substantially from other published descriptions of North American hemoparasites. We report a mean length for our *Lankesterella* sp. of 15.2 μ, compared to the 12.7 μ for *L. minima* (Barta and Desser, 1984) and the 10.1 μ reported by Clark et al. (1969). Indeed, the mean length we report exceeds the maximum length reported by Barta and Desser (1984); Clark et al. (1969) did not provide range or SD for their measurements.

The T*rypanosoma* sp. described in this study also differed from *T. boyli* (Leh-

TABLE 2. Mean dimensions of intracellular sporozoites of *Lankesterella* from selected North American ranid frogs.

Species	*n*	Length (±SD)	Range	Width (±SD)	Range
Lankesterella sp.[a]	245	15.2 (±1.5)	11–21	3.4 (±0.6)	1.5–5
Lankesterella minima[b]	10	12.7 (–)[d]	11.5–14.8	2.1 (–)	1.6–3.2
Lankesterella sp.[c]	(–)	10.1 (–)	(–)	2.2 (–)	(–)

[a] Obtained from *R. pretiosa* (this study).

[b] Obtained from *R. septentrionalis*, *R. clamitans*, and *R. catesbeiana* (Barta and Desser, 1984).

[c] Obtained from *R. cascadae* and *R. luteiventris*? (Clark et al., 1969).

[d] (–) = not available.

TABLE 3. Morphologic and morphometric comparison among *Trypanosoma* from selected North American ranid frogs.

Parasite structure[a]	Size (μm) or ratio				
	Trypanosoma sp.[b]	*T. boyli*[c]	*T. ranarum*[d]	*T. ranarum I*[e]	*T. ranarum II*[e]
PA	55.5	48.2	49.8	60.6	57.9
FF	8.1	0	14.3	13.5	13.2
PK	26.1	21.6	15.6	37.7	23.4
PN	32.5	28.2	21.8	41.8	28.6
NA	22.9	20.0	27.9	18.8	29.3
BW	13.4	10.3	18.2	4.7	8.2
KN	6.5	6.6	6.2	4.1	5.2
NL	6.9	4.7	n/a	3.0	3.6
NW	5.2	4.7	n/a	2.5	2.9
PK/PA	0.46	0.44	0.31	0.62	0.40
PN/PA	0.59	0.59	0.43	0.70	0.49
KN/PN	0.20	0.23	0.28	0.10	0.18
PK/PN	0.80	0.77	0.71	0.90	0.82
BW/PA	0.24	0.21	0.36	0.80	0.14
Kinetoplast	Circular	Rod-shaped	Square or rectangular	Rectangular or elliptical	Large square

[a] Abbreviations: FF = length of the free flagellum; PA = body length excluding the free flagellum; PK = distance from posterior end to the kinetoplast; PN = distance from the posterior end to the center of the nucleus; NA = distance from the center of the nucleus to the anterior end; BW = width of body at the level of the nucleus excluding the undulating membrane; KN = distance from the kinetoplast to the center of the nucleus; NL = length of the nucleus; NW = width of the nucleus.

[b] From *R. pretiosa* (this study).

[c] From *R. boylii* (Lehmann, 1959).

[d] From *R. catesbeiana, R. clamitans,* and *R. pipiens* (Woo, 1969).

[e] From *R. clamitans, R. esculenta, R. mugiens* and *R. pipiens* (Diamond, 1965).

mann, 1959d), which lacked a free flagellum but was similar in general size and shape to *T. ranarum* as described by Diamond (1965) and Woo (1969). However, the kinetoplast was small and circular and thus distinct from other published descriptions in which the kinetoplasts were square, rectangular, or rod-shaped (Table 3).

Frogs carrying either or both infections appeared vigorous and healthy. We observed no external physical or behavioral abnormalities associated with infected frogs. Considering the current status of this frog species, additional investigation of the impacts of infection are warranted, including analysis of weight:length ratios and the long-term survival of infected and uninfected individuals.

These findings, including careful measurements and organelle descriptions, may provide an important step in renewing the investigation of anuran blood parasites in the northwestern USA; such studies have received little attention over the past 30 yr. Description of other life stages of these parasites, identification of vectors (Desser et al., 1990), and molecular comparisons with previously described species are needed to provide accurate species designations (Desser, 2001). Determining ecological roles of these hemoparasites and their vectors will contribute to a better understanding of the basic biology of the Oregon spotted frog, a federal candidate for listing as an endangered species and currently state listed as a critical species by the Oregon Department of Fish and Wildlife (ONHP, 2001).

Voucher photographs of both parasites have been filed with the U.S. National Parasite Collection, as described by Bandoni (Bandoni and Duszynski, 1988):

USNPC #100099.00 (*Lankesterella* sp.); USNPC # 100100.00 (*Trypanosoma* sp.).

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