# Helminths of the Bullfrog, Rana catesbeiana (Ranidae), in California with Revisions to the California Anuran Helminth List

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Abstract.—Thirty-one bullfrogs, Rana catesbeiana, from northern California were examined for helminths. Gravid individuals representing three species of Trematoda, Glypthelmins quieta, Haematoloechus longiplexus and Megalodiscus temperatus; one species of Cestoda, Ophiotaenia magna; and three species of Nematoda, Cosmocercoides variabilis, Falcaustra catesbeianae and Oswaldocruzia pipiens were found. Larvae representing three species of Nematoda, Contracaecum sp., Eustrongylides sp., and Physaloptera sp. were also found. Rana catesbeiana is parasitized by generalist helminths that occur in other frogs and have previously been found in R. catesbeiana in other parts of North America. This is the first report of Falcaustra catesbeianae, Contracaecum sp. and Eustrongylides sp. from California anurans. The helminth host list for California anurans is revised.

The bullfrog, Rana catesbeiana, was first described from a collection taken in the vicinity of Charleston, South Carolina (Shaw, 1802). The original range covered most of eastern North America from the mouth of the Pecos River, Texas through the Panhandles of Texas and Oklahoma, extreme western Kansas, Nebraska and Minnesota, eastward to Maine and the northern half of Florida (Wright and Wright 1995). Subsequently, the bullfrog was introduced into each of the western states as well as Mexico and British Columbia, Canada (Stebbins 1985). In California, bullfrogs were first introduced in 1896 (Heard 1904) for human food after populations of native frogs, particularly the red-legged frog, Rana aurora, were overharvested (Jennings and Hayes 1985). Introductions and subsequent range expansions of the bullfrog have coincided with declines of native ranid frogs in western North America which has in turn generated interest in frog population ecology and competition (Kiesecker and Blaustein 1997; Kupferberg 1997; Lawler et al. 1999). However, little attention has been given to helminths of introduced frogs. To our knowledge, there are four reports of helminths in California bullfrogs (Ingles 1936; Nicol et al. 1985; Shields 1987; Wootton et al. 1993). Helminths of the bullfrog in North America have been summarized by Andrews et al. (1992). Additional helminths are listed in Bursey and DeWolf (1998), Goldberg et al. (1998) and McAlpine and Burt (1998). The purpose of this paper is to report additional helminths of California bullfrogs and to revise the helminth list for California anurans.

#### Methods

Thirty-one bullfrogs collected in 1997 and 1998 from northern California were examined for helminths. All frogs were deposited in the herpetology collection of the Natural History Museum of Los Angeles County (LACM) after examination. Sixteen frogs (LACM 144342-144357) were from Upper Searsville Lake, Jasper Ridge Biological Preserve, Woodside, San Mateo County (37°30'N, 122°30′W); 15 frogs (LACM 146746-146751, 146753, 146754, 146756-146762) were from sites between Uvas and Calero Reservoirs, Santa Clara County (37°05'N, 121°45'W). The frogs were initially fixed in 10% formalin and preserved in 70% ethanol. The body cavity was opened by a longitudinal incision from throat to pelvis and the gastrointestinal tract, lungs and urinary bladder were removed. Each organ was opened and examined for helminths under a dissecting microscope. The surface of the liver and the body cavity were also searched. Nematodes were placed on a microscope slide and cleared in glycerine. A coverslip was added to the slide and the nematode was identified using a compound microscope. Cestodes and trematodes were rehydrated, stained in hematoxylin, dehydrated in a series of graded ethanols, cleared in xylene, mounted on a glass slide in Canada balsam and identified using a compound microscope.

Three similarity indices were calculated in order to compare the *R. catesbeiana* helminths from San Mateo and Santa Clara Counties. The Jaccard coefficient is based on species presence in a community and ranges from 0 (no species in common) to 1.0 (all species in common); Morisita's index considers number of species, number of individuals, and proportion of the total represented by each species and ranges from 0 (no similarity) to 1.0 (identical); percent similarity is based on species abundance and ranges from 0 (no similarity) to 100 (same species found in both communities at similar abundances) (Brower et al. 1997).

#### Results

Gravid individuals of three species of Trematoda, Glypthelmins quieta (Stafford, 1900), Haematoloechus longiplexus Stafford 1902, Megalodiscus temperatus (Stafford, 1905); one species of Cestoda, Ophiotaenia magna Hannum, 1925; and three species of Nematoda, Cosmocercoides variabilis (Harwood, 1930), Falcaustra catesbeianae Walton, 1929, Oswaldocruzia pipiens Walton, 1929, were found. Larvae representing three species of Nematoda, Contracaecum sp., Eustrongylides sp., and Physaloptera sp. were also found. Prevalence (percent of sample infected) and mean intensity (mean number of helminths per infected frog ± 1 SD) by helminth species are given in Table 1.

The helminths exhibited site specific infections: Glypthelmins quieta, Ophiotaenia magna, Cosmocercoides variabilis and Oswaldocruzia pipiens were found in the small intestine, Megalodiscus temperatus and Falcaustra catesbeianae in the large intestine, and Haematoloechus longiplexus in the lungs. Larvae of Contracaecum sp., and Eustrongylides sp. were found in cysts within the body cavity and most often attached to the mesenteries; larvae of Physaloptera sp. were found within the lumen of the stomach. In no cases did two parasite species occupy the same site in a single host.

Selected helminths were deposited in the United States National Parasite Collection, USNPC, Beltsville, Maryland 20705 as: *Glypthelmins quieta* (91248),

Table 1. Prevalence (as $\%$ ), mean intensity $\pm$ 1 SD, and range for helminths from <i>Rana catesbeiana</i>
from San Mateo and Santa Clara Counties, California; n = number of hosts examined.

entesSpa vactorquid		an Mateo Cou n = 16 109 mm ± 1			anta Clara Cou n = 15 153 mm ± 1	
Rocte College State	Preva- lence	Mean intensity	Range	Preva- lence	Mean intensity	Range
Glypthelmins quieta	6	1		7	12	1. 1
Haematoloechus longiplexus	56	$4.8 \pm 5.2$	1-17	49	$3.7 \pm 5.1$	1-17
Megalodiscus temperatus	56	$3.2 \pm 3.5$	1-12		MARTINE.	
Ophiotaenia magna	_			20	$1 \pm 0$	-
Cosmocercoides variabilis	_		-	7	2	and <del>-</del>
Falcaustra catesbeianae	50	$4.3 \pm 3.6$	1-12	_		_
Oswaldocruzia pipiens	_	_	_	7	6	_
Contracaecum sp. (larvae)	38	$2.3 \pm 1.4$	1-5	_	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	-
Eustrongylides sp. (larvae)	14-11			20	$1 \pm 0$	
Physaloptera sp. (larvae)	11-20	Bolo - man	313-200	20	$2.7 \pm 1.5$	1-4

Haematoloechus longiplexus (91244), Megalodiscus temperatus (91245), Ophiotaenia magna (91249), Cosmocercoides variabilis (91250), Falcaustra catesbeianae (91246), Oswaldocruzia pipiens (91251), Contracaecum sp. (91247), Eustrongylides sp. (91252), Physaloptera sp. (91253).

#### Discussion

All helminths found in this study have previously been reported from *Rana catesbeiana* in other parts of its range as well as from other ranids (Dyer 1991; Andrews et al. 1992). However, this is the first report of *Falcaustra catesbeianae*, *Contracaecum* sp. (larvae) and *Eustrongylides* sp. (larvae) from California anurans (Table 2).

Glypthelmins quieta, Haematoloechus longiplexus and Megalodiscus temperatus are common trematode parasites of North American frogs (Smyth and Smyth 1980). These three species require a molluscan first intermediate host. After release from the molluscan host, cercariae of Glypthelmins quieta and Megalodiscus temperatus penetrate the skin of anurans and encyst beneath the epidermis. Infection occurs when a frog ingests its own cast skin after molting. Cercariae of Haematoloechus longiplexus penetrate and encyst in naiads of dragonflies; infection occurs through ingestion of dragonflies (Smyth and Smyth 1980). The host list for Glypthelmins quieta includes five genera of anurans, Acris, Bufo, Hyla, Pseudacris and Rana; for Haematoloechus longiplexus, two genera, Bufo and Rana; for Megalodiscus temperatus, four genera of anurans, Bufo, Hyla, Pseudacris, and Rana, five genera of Caudata, Ambystoma, Amphiuma, Desmognathus, Notophthalmus and Pseudotriton, and one genus of Serpentes, Coluber (Parker 1941; Catalano et al. 1982; Prudhoe and Bray 1982).

There is some confusion surrounding the identity of North American ranid proteocephalid cestodes; four have been reported, namely, *Ophiotaenia magna*, *Ophiotaenia saphena* Osler, 1931, *Crepidobothrium olor* Ingles, 1936 and *Ophiotaenia gracilis* Jones, Cheng and Gillespie, 1958. Brooks (1978) discussed morphological characteristics of these species and concluded no significant morpho-

Table 2. Helminths of California Anura.

Helminth	County	Reference
Trematoda	3	
Alaria mustelae Bosma, 1931		
Hyla regilla	Santa Clara	Johnson et al. 1999
Alaria sp. (mesocercaria)		
Hyla cadaverina	Riverside	Goldberg and Bursey 2001a
Hyla regilla	Santa Clara	Goldberg and Bursey 2001b
Brachycoelium lynchi Ingles, 1935		
Rana aurora	Siskiyou	Ingles 1936
Cephalogonimus americanus Stafford, 1902		
= Cephalogonimus brevicirrus Ingles, 1932 = Cephalogonimus retusus of Walton, 1938		
Rana aurora	Kern	Ingles 1932a
Rana aurora	Kern	Ingles 1936
Clinostomum sp. (metacercaria)		1
Hyla regilla	Humboldt, Santa Clara	Goldberg and Bursey 2001a
Rana aurora	not given	Ingles 1936
Rana boylii	not given	Ingles 1936
Fibricola sp. (metacercaria)		
Hyla cadaverina	Los Angeles	Goldberg and Bursey 2001a
Glypthelmins sp.		
Bufo boreas	Los Angeles	Koller and Gaudin 1977
Glypthelmins quieta (Stafford, 1900) Stafford, 1905		
= Distomum quietum Stafford, 1900		
= Margeana californiensis Cort, 1919		
= Glypthelmins californiensis (Cort, 1919) Miller, 1930		
= Glypthelmins subtropica Harwood, 1932		
Rana aurora referred by Sullivan, 1976	San Francisco	Cort 1919

Table 2. Continued.

Helminth	County	Reference
Rana aurora referred by Sullivan, 1976	San Diego, Butte	Ingles 1936
r	Butte	Ingles 1936
Rana boylii referred by Sullivan, 1976	Marin, Sonoma	Lehmann 1960
eia	San Mateo, Santa Clara	this study
Glypthelmins shastai Ingles, 1935		
Bufo boreas	Shasta	Ingles 1936
Gorgoderina sp.		
Bufo boreas	Siskyou	Ingles 1936
Hyla cadaverina	Los Angeles, Orange	Goldberg and Bursey 2001a
Rana aurora	Kern	Ingles 1932a
Gorgoderina aurora Ingles, 1935		
Rana aurora	San Francisco, San Joaquin	Ingles 1936
Gorgoderina multilobata Ingles and Langston, 1933		
Rana aurora	Los Angeles	Ingles and Langston 1933
Rana aurora	Butte	Ingles 1936
Rana boylii	Butte	Ingles and Langston 1933
Rana boylii	Butte	Ingles 1936
Rana pretiosa	Butte	Ingles 1936
Gyrodactylus catesbeianae Wootton, Ryan, Demaree and Critchfield, 1993		
Rana catesbeiana (tadpoles)	Glenn	Wootton, et al. 1993
Haematoloechus sp.		
Rana aurora	Kern	Ingles 1932a
Rana aurora	Marin, Sonoma	Lehmann 1960
Rana boylii	Marin, Sonoma	Lehmann 1960
Haematoloechus complexus (Seely, 1906) Yamaguti, 1958		
= Pneumonoeces complexus Seely, 1906		
= Haematoloechus confusus Ingles, 1932		
= Haematoloechus oxyorchis Ingles, 1932		

Table 2. Continued.

Helminth	County	Reference
= Ostiolum oxyorchis (Ingles, 1932) Ingles, 1936	7,10	
Rana aurora	San Francisco	Ingles 1932b
Rana aurora	Los Angeles	Ingles 1933
Rana aurora	San Francisco	Ingles 1936
Haematoloechus kernensis Ingles, 1932		
= Haematoloechus tumidus Ingles, 1932		
Bufo boreas	San Bernardino	Goldberg et al. 1999
Rana aurora	Kern	Ingles 1932b
Rana aurora	Kern	Ingles 1936
Haematoloechus longiplexus Stafford, 1902		
Rana catesbeiana	Ventura	Shields 1987
Rana catesbeiana	San Mateo, Santa Clara	this study
Haematoloechus variplexus Stafford, 1902		
= Haematoloechus buttensis Ingles, 1936		
Rana boylii	Butte	Ingles 1936
Halipegus aspina Ingles, 1932		
Rana boylii	Butte	Ingles 1936
Rana boylii	Marin, Sonoma	Lehmann 1960
Langeronia burseyi Dailey and Goldberg, 2000		
Hyla cadaverina	Orange	Dailey and Goldberg 2000
Hyla cadaverina	Orange	Goldberg and Bursey 2001a
Levinseniella ophidea Nicol, Demaree and Wootton, 1985		
Rana catesbeiana	Lassen	Nicol et al. 1985
Megalodiscus microphagus Ingles, 1936		
Bufo boreas	Shasta	Ingles 1936
Megalodiscus temperatus (Stafford, 1905) Yamaguti, 1958		
Rana boylii	Marin, Sonoma	Lehmann 1960

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Helminth	County	Reference
Rana catesbeiana Rana catesbeiana	Butte San Mateo	Ingles 1936 this study
Ribeiroia sp. (metacercaria)		
Bufo boreas	Santa Clara	Johnson et al. 1999
Hyla regilla	Santa Clara	Johnson et al. 1999
Rana catesbeiana	Santa Clara	Johnson et al. 1999
Cestoda		
Cylindrotaenia americana Jewell, 1916		
Bufo canorus	Mariposa	Walton 1941
Distoichometra bufonis Dickey, 1921		
Bufo boreas referred by Goldberg et al., 1999	Los Angeles	Koller and Gaudin 1977
Bufo boreas	Los Angeles, San Bernardino	Goldberg et al. 1999
Hyla cadaverina	Riverside	Goldberg and Bursey 2001a
Hyla regilla referred by Goldberg et al., 1999	Los Angeles	Koller and Gaudin 1977
Hyla regilla	Santa Clara	Goldberg and Bursey 2001b
Ophiotaenia magna Hannum, 1925		
= Ophiotaenia saphena Osler, 1931		
= Crepidobothrium olor Ingles, 1936		
= Ophiotaenia gracilis Jones, Cheng and Gillespie, 1958		
Rana aurora	Alameda	Ingles 1936
Rana catesbeiana	Santa Clara	this paper
Unidentified dilepinid		
Rana boylii	Siskiyou	Ingles 1936
Nematoda		
Aplectana itzocanensis Bravo Hollis, 1943		
Bufo boreas	Orange, Riverside, San Bernardino	Goldberg et al. 1999
Contracaecum sp. (larvae)		
Rana catesbeiana	San Mateo	this study

Table 2. Continued.

Helminth	County	Reference
Cosmocercoides variabilis (Harwood, 1930) Travassos, 1931		
= Cosmocerca dukae Holl, 1928 (in part) = Oxysomatium americana Walton, 1929 = Oxysomatium variabilis Harwood, 1930		
Bufo boreas referred by Goldberg et al., 1999	Shasta	Ingles 1936
Bufo boreas referred by Goldberg et al., 1999	Los Angeles	Koller and Gaudin 1977
Bufo boreas	Riverside	Goldberg et al. 1999
Bufo canorus	Mariposa	Walton 1941
Hyla regilla referred by Goldberg et al., 1999	Los Angeles	Koller and Gaudin 1977
Rana aurora referred by Goldberg et al., 1999	Butte	Ingles 1936
Rana catesbeiana	Kern	Ingles 1936
Rana catesbeiana	Santa Clara	this study
Eustrongylides sp. (larvae)		
Rana catesbeiana	Santa Clara	this study
Falcaustra catesbeianae Walton, 1929		
Rana catesbeiana	San Mateo	this study
Falcaustra inglisi (Anderson, 1964) Baker, 1980		
= Oxysomatium inglisi Anderson, 1964		
Bufo boreas	Los Angeles	Goldberg et al. 1999
Falcaustra pretiosa (Ingles, 1935) Freitas and Lent, 1941		
= Spironoura pretiosa Ingles, 1935		
Bufo boreas	Inyo	Walton 1941
Rana aurora	Santa Clara	Walton 1941
Rana boylii	Tuolumne	Walton 1941
Rana pretiosa	Plumas	Ingles 1936
Falcaustra ranae (Walton, 1941) Chabaud and Golvan, 1957		
= Spironoura ranae Walton, 1941		
Rana boylii	Mariposa	Walton 1941

Table 2. Continued.

Helminth	County	Reference
Oswaldocruzia pipiens Walton, 1929		
= Oswaldocruzia waltoni Ingles, 1935		
Bufo boreas	Butte, Kern, San Diego	Ingles 1936
Bufo boreas referred by Goldberg et al., 1999	Los Angeles	Koller and Gaudin 1977
Bufo boreas	Los Angeles, Riverside	Goldberg et al. 1999
Hyla regilla referred by Goldberg et al., 1999	Los Angeles	Koller and Gaudin 1977
Hyla regilla	Santa Clara, Imperial, Los Angeles,	Goldberg and Bursey 2001b
	Orange, Riverside	
Rana aurora	Butte, Kern, San Diego	Ingles 1936
Rana catesbeiana	Santa Clara	this study
Physaloptera sp. (larvae)		
Bufo boreas	Orange	Goldberg et al. 1999
Hyla cadaverina	Los Angeles, Orange	Goldberg and Bursey 2001a
Hyla regilla	Orange	Goldberg and Bursey 2001b
Rana catesbeiana	Santa Clara	this study
Rhabidas sp.		
Bufo boreas	not given	Ingles 1936
Bufa boreas	Los Angeles	Koller and Gaudin 1977
Hyla regilla	Los Angeles	Koller and Gaudin 1977
Rana boylii	not given	Ingles 1936
Rana boylii	Marin, Sonoma	Lehmann 1960
Rhabdias americanus Baker, 1978		
Bufo boreas	San Bernardino	Goldberg et al. 1999
Rhabdias joaquinensis Ingles, 1935		
Rana aurora	San Joaquin	Ingles 1936
Rhabdias ranae Walton, 1929		
Hyla cadaverina	Los Angeles, Riverside	Goldberg and Bursey 2001a
Hyla reailla	Oursell Discoulds	Coldbone and Dimensi 2001b

logical differences existed between them. He did not place them in synonymy but assigned all to *Proteocephalus*. More recently, Schmidt (1986) assigned these species to *Ophiotaenia*. Since it is not possible to distinguish these four species and because we can find no difference between individuals collected in California and Ohio (see Bursey and DeWolf 1998), we have assigned our specimens to *Ophiotaenia magna* which has priority. In addition, we have referred all California ranid proteocephalids to *Ophiotaenia magna* (Table 2).

Cosmocercoides variabilis, Falcaustra catesbeianae and Oswaldocruzia pipiens are common nematode parasites of North American frogs (Baker 1987). Like the trematodes found in this study, these nematodes are generalists, i.e., found in more than one host. However, some uncertainty exists for hosts of North American species of Cosmocercoides. Cosmocercoides variabilis, originally described as Oxysomatium variabilis by Harwood (1930) from Bufo valliceps collected at Houston, Texas was considered a synonym of the molluscan parasite Cosmocercoides dukae by Ogren (1953, 1959) who presumed that amphibians acquired C. dukae infections by ingesting infected molluscs. Cosmocercoides dukae was first described as Cosmocerca dukae by Holl (1928) from Triturus viridescens collected in North Carolina. Wilkie (1930) established the genus Cosmocercoides, and Travassos (1931) included both C. dukae and C. variabilis in his monograph on the Cosmocercidae. Vanderburgh and Anderson (1987) demonstrated that these two species of Cosmocercoides are distinct. The major difference between the two species is the number of rosette papillae of the male; C. dukae with 12 pairs; C. variabilis with 14 to 20. Specimens collected in this study exhibited 16–18 rosette papillae. The host list for C. variabilis includes the five genera of anurans, Bufo, Gastrophryne, Hyla, Pseudacris, Rana, two genera of Caudata, Ambystoma, Notophthalmus; two genera of lizards, Scincella, Ophisaurus; three of snakes, Heterodon, Micrurus, Storeria; and one of tortoises, Terrapene (Baker 1987). Ingles (1936) reported C. dukae from Taricha torosa, Rana aurora and Bufo boreas from California but illustrated 16 papillae and for this reason we have referred his specimens to Cosmocercoides variabilis (Table 2). Falcaustra catesbeianae has been reported from four genera of Anura, namely, Gastrophryne, Hyla, Pseudacris, Rana, and two genera of Caudata, Siren and Typhlotriton (Baker 1987). All North American specimens of the genus Oswaldocruzia have been referred to O. pipiens by Baker (1977). This species is widely distributed in North America and has been reported from six genera of anurans, Acris, Bufo, Hyla, Pseudacris, Rana, Scaphiopus; three of Caudata, Desmognathus, Eurycea, Plethodon, seven genera of lizards, Anolis, Eumeces, Elgaria, Gerrhonotus, Heloderma, Sceloporus, Scincella; and one of tortoises, Terrapene (Baker 1987; Goldberg and Bursey 1991).

Three species of nematodes not reaching maturity in frogs were present: Contracaecum sp., Eustrongylides sp. and Physaloptera sp. Species of these genera require intermediate hosts: Contracaecum, aquatic invertebrates; Eustrongylides, aquatic oligochaetes; Physaloptera, terrestrial insects (Anderson 2000). The definitive hosts of species of Contracaecum are piscivorous birds and aquatic mammals, species of Eustrongylides are limited to piscivorous birds, and species of Physaloptera parasitize mammals, birds and reptiles (Anderson 2000). Because individuals of Contracaecum and Eustrongylides were found in cysts, the possibility of Rana catesbeiana as a paratenic host must be considered. The absence

of *Physaloptera* in cysts suggests that they are taken with insect prey but cannot establish infection and are soon excreted.

Interestingly, of the ten species of helminths found in this study, only two trematodes, *Glypthelmins quieta* and *Haematoloechus longiplexus* were found at both locations of bullfrog collections. The 16 bullfrogs from San Mateo harbored 94 individuals representing 5 helminth species; 15 bullfrogs from Santa Clara harbored 67 individuals representing seven helminth species. The calculated results for Jaccard coefficient, Morisita's index and percent similarity were 0.2, 0.7, 47.9, respectively, indicating that the helminth communities harbored by the two bullfrog populations were not similar in structure. Other studies of California anurans (see Koller and Gaudin 1977; Goldberg and Bursey 2001a, 2001b) from multiple localities have reported similar results, i.e., spotty distribution of helminths for a particular host species.

The anuran helminths listed in Table 1 are generalists in that they are capable of infecting a number of hosts. Thus, it is possible that a particular host is unimportant; infection in a particular host may fluctuate from location to location, but the helminth population maintains an overall presence. Given the earlier introduction of the bullfrog to California and the later helminthological surveys (Table 2), it is not possible to determine whether bullfrogs acquired these helminths in California or transported them into the state. Neither is it possible to gauge the breadth of helminth infection for California anurans because only 8 (35%) of the 23 species of anurans known to occur in California (Stebbins 1985) have been examined for helminths. Examination of additional California anuran species must occur before the helminth community of these hosts can be assessed.

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