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Oral Chytridiomycosis in the Mountain Yellow-Legged Frog (*Rana muscosa*)

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The chytrid fungus *Batrachochytrium dendrobatidis* was originally reported in wild frog populations in Panama and Australia, and from captive frogs in the U.S. National Zoological Park (Washington, DC). This recently described fungus affects the keratinized epidermis of amphibians and has been implicated as a causative factor in the declines of frog populations. We report here the presence of *B. dendrobatidis* in larval and recently metamorphosed mountain yellow-legged frogs (*Rana muscosa*) in or near the Sierra Nevada Mountains of California, an area where declines have been documented in all five species of native anurans. Forty-one percent (158 of 387) of larval *R. muscosa* examined in the field with a hand lens and 18% (14 of 79) of preserved larvae had abnormalities of the oral disc. Twenty-eight larvae were collected from 10 sites where tadpoles had been observed with missing or abnormally keratinized mouthparts, and 24 of these were examined for infection. Sixty-seven percent (16 of 24) of these tadpoles were infected with *B. dendrobatidis*. *Batrachochytrium dendrobatidis* was cultured from both tadpoles and recent metamorphs from one of these sites. Tadpoles with mouthpart abnormalities or confirmed chytrid fungus infections were collected at 23 sites spanning a distance of > 440 km and an elevational range from 1658–3550 m. Life-history traits of *R. muscosa* may make this species particularly susceptible to infection by *Batrachochytrium*. We recommend that biologists examine tadpoles for oral disc abnormalities as a preliminary indication of chytridiomycosis. Further, we believe that biologists should take precautions to prevent spreading this and other amphibian diseases from one site to another.

SIGNIFICANT declines of amphibian populations have been reported from many areas around the world. Some of the best-documented declines have occurred in the Sierra Nevada, Cascade, and Rocky Mountains of the western United States (Carey, 1993; Fellers and Drost, 1993; Drost and Fellers, 1996). In the Sierra Nevada, declines have been documented in all five species of native anurans. The causative factors are difficult to determine because most declines have taken place when amphibians were not being monitored. Investigators have suggested increased levels of ultraviolet-B radiation (Blaustein et al., 1994; Keisecker and Blaustein, 1995), introduction of nonnative fish (Bradford et al., 1993; Fellers and Drost, 1993), disease (Bradford, 1991), air-borne dispersal of insecticides and herbicides from agricultural areas (Datta et al., 1998; McConnell et al., 1998; LeNoir et al., 1999), and alteration of habitat resulting from the suppression of natural fires (Fellers and Drost, 1993) as possible factors.

The chytrid fungus *Batrachochytrium dendrobatidis* was first reported in wild frog populations in Panama and Australia (Berger et al., 1998; Lips, 1999). The fungus was present in populations that were undergoing dramatic declines. Since then, this fungus has been reported in more than 75 wild-caught species of amphibians worldwide. Most of these are from Australia (47 species, 14 genera), but there are now reports from Europe (2, 2), Africa (2, 2), South America (2, 2), Central America (6, 5), and North America (15, 5), but none from Asia (R. Speare and L. Berger, http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyglob.htm; GMF and DEG, unpubl.). This distribution of chytrid-infected amphibians may reflect the extent to which biologists have looked on the different continents as much as it reflects the actual distribution of the fungus.

Chytrid is the common name for fungi in the phylum Chytridiomycota that has one class (Chytridiomycetes) and over 100 genera. Members of this group of microscopic, zoospore-producing fungi are widespread in soil and water where most are decomposers of cellulose and other plant material, chitin, and keratin (Sparrow, 1960; Powell, 1993). Usually, each species of chytrid has only one preferred substrate, although some species are generalists. Chytrids also are known as parasites of other fungi, algae, higher plants, protozoa, and invertebrates, but before 1998, none had been reported to infect vertebrates (Berger et al., 1998). Histologically and ultrastructurally, the chytrids from Pana-

manian, Australian, and captive amphibians are indistinguishable (Longcore et al., 1999). Taxonomically important ultrastructural features of the chytrid zoospores from the Australian, Panamanian, and captive amphibians suggest that the same, or closely related, organisms are present at all sites (Longcore et al., 1999). Based on an isolate from the U.S. National Zoological Park, Longcore et al. (1999) described and named the chytrid as a new genus and species, *B. dendrobatidis* that grows in the keratinized epidermal cells of amphibians.

The oral discs of tadpoles (terminology as recommended by McDiarmid and Altig, 1999) normally have keratinized jaw sheaths (beaks) and toothrows that are heavily pigmented. In healthy tadpoles, these structures are conspicuously black and bilaterally symmetrical. Most tadpole species also have labial papillae along the margins of the oral disc (McDiarmid and Altig, 1999). In Central American tadpoles infected with *Batrachochytrium* fungus, all these structures were abnormally formed or lacking pigment. Because *Batrachochytrium* infection (chytridiomycosis) has been reported in populations of declining amphibians in the United States, we examined the oral discs of live and preserved tadpoles of mountain yellow-legged frogs (*Rana muscosa*) in the Sierra Nevada Mountains of California to determine whether fungal infections might be present. Our finding of oral disc abnormalities and the presence of chytrid fungus add significantly to our understanding of disease. Our findings also signal the need to modify field techniques to decrease the chances of spreading a potentially lethal fungus in a region with many active herpetologists and other biologists.

Materials and Methods

Study area and site selection.—Tadpoles were examined from sites throughout much of the Sierra Nevada Mountains of California, as well as one site in the Cascade Mountains immediately to the north and one site at the edge of the Great Basin to the east. Sites spanned a distance of $>$ 440 km and an elevational range from 1658–3550 m (Appendix 1), covering nearly the entire geographic and elevational range of the Sierran subspecies of *R. muscosa*.

Sites were selected and surveyed as part of another study designed to evaluate the status of declining amphibians in California. Site selection was not based on the likelihood of finding (or not finding) chytrid fungus or the likelihood of finding amphibians.

Gross examinations.—Tadpoles were examined initially in the field with a 10× hand lens. Tadpoles that were collected were examined in the laboratory using a dissecting microscope. Normal toothrows form a nearly symmetrical arch across the upper or lower portions of the oral disc (McDiarmid and Altig, 1999). Tadpoles of *R. muscosa* typically have anterior and posterior jaw sheaths (upper and lower beaks), 2–4 anterior (upper) toothrows, and four posterior (lower) tooth rows (Stebbins, 1985). The toothrows and jaw sheaths are formed from keratin, and normally are heavily pigmented with melanin (black). Often the innermost 1–2 rows of the anterior (upper) toothrows are disjunct in the midline and form bilaterally symmetrical segments. Tadpoles with toothrow abnormalities typically were missing some or all pigmented portions of the toothrow; some toothrows had irregular (asymmetrical) gaps (i.e., loss of multiple individual labial teeth). Missing toothrows (or portions thereof) could be recognized because a raised ridge of depigmented, white or pinkish-white tissue remained. Although we did not stage tadpoles in the field, it was generally possible to assess *R. muscosa* mouthparts from Gosner stage 25 through stage 40 (Gosner, 1960), especially because the larvae are relatively large.

Existing collections of preserved tadpoles were also examined with a 10× hand lens, and occasionally with a dissecting microscope at 20–30×. Thirty-six tadpoles collected for a DNA study (GMF, unpubl.) and 43 tadpoles at the Museum of Vertebrate Zoology, University of California at Berkeley were examined. Specimens for the DNA study were individually preserved in 95% ethanol. Museum specimens were stored in 70% ethanol.

Histological examinations were performed on 28 tadpoles and one recent metamorph from 10 sites where oral disc abnormalities were observed. Because sections from one tadpole resulted in equivocal results and suitable sections were not obtained from three additional tadpoles, our histological analysis includes 24 tadpoles. Tadpoles were collected in the field and shipped via overnight courier to Madison, Wisconsin. Upon receipt, tadpoles were sedated in tricaine methanesulfonate (MS-222; Sigma-Aldrich, St. Louis, MO) and oral discs were examined under a dissecting microscope. Swabs (Mini-tip Culturette, Becton Dickinson and Company, Cockeysville, MD) for bacterial cultures were collected from the mouths, body cavities, intestines, and livers. Portions of livers, fat bodies, and mesonephroi (''kidneys'') were collected and promptly frozen at −70 C for viral cultures. Carcasses were fixed by immersion in

McDowell-Trump universal fixative (buffered 10% formalin with 1% glutaraldehyde). Tissues were processed through graded concentrations of ethanol and xylene, embedded in paraffin and cut into six micron-thick sections. Hematoxylin and eosin (H&E), Giemsa, Gomori's methenamine silver (GMS), periodic acid-Schiff (PAS), and Steiner's silver stains were used to examine tissues and fungi.

We cultured tissues for viruses from three tadpoles from each of three sites (Y-321, Y-764, and Y-1579). Tissues were cultured using turtle heart cells (THC), Chinook salmon embryo cells (ChSE), and fathead minnow cells (FM). Frozen tissues were mixed with 3 ml of Leibovitz's L-15 medium with 2% fetal calf serum (20% weight/volume) containing 500 μg/ml gentamycin (Sigma Chemical Company, Saint Louis, MO), 800 μg/ml streptomycin, 10 μg/ml fungizone, and 800 units/ml penicillin (JRH Biosciences, Lexena, KS). After two hours incubation at room temperature (22–25 C), the suspension was centrifuged at 3000 × G for 20 min. Supernatant fluids were harvested, diluted 1:10, 1:50, and 1:500, and then 0.1 ml of each dilution was inoculated onto cell lines grown in 48-well microliter plates and incubated at 15 C. If no cytopathic effect (CPE) was observed in 10 days, fluids were removed from the wells and used to inoculate fresh cell monolayers (one blind passage). If no CPE developed after an additional 10 days, the samples were considered negative for virus.

The same nine tadpoles used for viral cultures were examined for bacteria. Bacterial swabs were streaked onto ovine blood agar and brain-heart infusion media. Special cultures of the intestines for *Salmonella, Campylobacter,* and *Yersinia* were performed on one pool of intestines from the three sites sampled.

For cultures of chytrid fungus, four live tadpoles and two recent metamorphs were collected 17 October 1998 from site Y-764 where abnormal oral discs had been observed. Small pieces of the toothrows and jaw sheaths from the tadpoles were first examined in distilled water under a compound microscope to verify the presence of chytrid fungi. Epidermal tissue pieces that contained profiles of spherical fungal thalli (Pessier et al., 1999) were cut into 1–2 mm^2 pieces and placed on 1% agar plates. Each piece of tissue was then pushed and dragged through the agar with a sterile needle to remove bacteria and yeast. Cleaned bits of tissue were moved to a clean plate of mTGhL nutrient agar (0.8% tryptone, 0.2% gelatin hydrolysate, 0.4% lactose, 1% agar, with 0.02% penicillin-G, and 0.04% streptomycin sulfate added after autoclaving). After establishment of growth, colonies were transferred to TGhL agar (1.6% tryptone, 0.4% gelatin hydrolysate, 0.2% lactose, and 1% agar). Although the skin of the hindlimb digits and webs of the recent metamorphs were infected with chytrids, the oral discs contained more active fungal thalli and were used to isolate *Batrachochytrium.*

Results

Field observations.—During 1998–2000, 387 live tadpoles were examined in the field for abnormalities of the oral discs (e.g., toothrows, jaw sheaths, and labial papillae; Table 1). Abnormalities of the oral discs were found at 16 of 23 (70%) of sites. At sites with abnormal tadpoles, abnormalities were present in 4.2–100% of each population, with a mean prevalence of 40.8% and a median of 62.5%. Figure 1A–B illustrates *R. muscosa* tadpoles with missing toothrows, depigmented jaw sheaths, and swollen and reddened labial papillae.

Examinations of preserved specimens.—Of the preserved tadpoles, 14 of 36 (38.9%) recently collected *R. muscosa* tadpoles, and none of the 43 *R. muscosa* tadpoles from the Museum of Vertebrate Zoology, had obvious abnormalities (Table 1). If one compares the recent collections (1993–1999) with the older, museum collections (1955–1976), the observed differences in frequency of abnormalities is highly significant ($\chi^2 = 20.32$, $P < 0.0001$).

Necropsy examinations.—Except for oral disc abnormalities and a thickening of the epidermis at the toe tips in one late stage tadpole and in one recent metamorph, all 28 tadpoles and the metamorph appeared normal and healthy. They had large yellow fat bodies, abundant food in their gastrointestinal tracts, and no histological evidence of significant infectious disease in internal organs.

Tadpoles from sites Y-764 and Y-321 showed oral disc abnormalities that included (1) a loss of pigmentation in the toothrows, (2) lost, interrupted, or misshaped toothrows, and (3) swollen and pinkish or reddish labial papillae (Fig. 1A–B). The loss of black pigment on the upper and lower jaw sheaths ranged from mild to severe, ranging from 10–100% per tadpole (median, 70%; mean, 60%, $n = 7$) and consisted of segments of the jaw sheaths that were distinctly white or pinkish-white instead of the normal jet-black condition. Four tadpoles from site Y-1579 had normal jaw sheaths. Toothrows of tadpoles from this site were also affected. Loss of toothrow pigmentation ranged from a relatively subtle

TABLE 1. LIVE AND PRESERVED *Rana muscosa* LARVAE EXAMINED FOR ABNORMAL ORAL DISCS (JAW SHEATHS, TOOTHROWS, AND LABIAL PAPILLAE). Four tadpoles from site Y-764 collected on 15 September 1998 had chytrid infections confirmed by culturing.

	Field		Laboratory		Histological		
Site	Tadpoles examined	Number abnormal	Tadpoles examined	Number abnormal	Tadpoles examined	Number abnormal	Date
L-553	4	1	—	—	—	—	08/24/1998
S-294	—	—	4	0	—	—	08/29/1993
S-309	12	4	—	—	—	—	09/03/1998
S-376	—	—	5	0	—	—	09/24/1993
S-387	—	—	5	0	—	—	09/25/1999
S-545	16	6	—	—	—	—	09/03/1998
S-562	—	—	1	0	—	—	07/24/1994
S-568	20	0	—	—	—	—	09/03/1998
T-083	5	0	—	—	—	—	08/03/1998
T-083	20	0	—	—	—	—	07/25/2000
T-510	32	2	—	—	—	—	09/08/2000
Y-029	—	—	2	2	2	2	07/04/1998
Y-029	14	6	—	—	—	—	06/01/1999
Y-029	1	1	—	—	—	—	05/24/2000
Y-258	—	—	1	1	—	—	07/25/1993
Y-258	15	0	1	0	1	0	08/20/1998
Y-321[a]	22	18	1	1	3[b]	3	09/17/1998
Y-638	—	—	1	0	—	—	08/18/1994
Y-764	16	10	9	6	12	9	09/15/1998
Y-764[a,c]	16	16	—	—	—	—	10/17/1998
Y-764B	35	35	—	—	—	—	05/25/1999
Y-765	15	0	—	—	—	—	09/15/1998
Y-765	10	10	—	—	—	—	05/25/2000
Y-987	18	18	—	—	—	—	09/17/1998
Y-1025	—	—	1	1	—[d]	0	05/26/1999
Y-1219	6	0	—	—	—	—	09/21/1998
Y-1532	24	1	1	1	1	0	07/21/1998
Y-1539	7	0	—	—	—	—	07/29/1998
Y-1542	10	0	—	—	—	—	07/29/1998
Y-1566	5	1	—	—	—	—	08/02/1998
Y-1579	9	9	—	—	—	—	08/03/1998
Y-1579[a]	10	7	1	0	2[e]	0	09/16/1998
Y-1608	9	0	—	—	—	—	08/19/1998
Y-1610	15	7	—	—	—	—	08/19/1998
Y-1665	20	5	—	—	—	—	08/23/1998
Y-1849	1	1	1	1	1	0	08/27/1999
EWA-1	—	—	1	1	1	1	07/15/1999
EWA-2	—	—	1	0	1	1	07/16/1999
MVZ-2	—	—	4	0	—	—	06/08/1955
MVZ-3	—	—	12	0	—	—	07/14/1961
MVZ-4	—	—	11	0	—	—	05/04/1968
MVZ-5	—	—	16	0	—	—	06/20/1976
Total Individuals	387	158 (40.8%)	79	14 (17.7%)	24	16 (66.7%)	
Total sites	23	16 (69.6%)	19	8 (42.1%)	9	5 (55.6%)	

[a] Tissue was cultured from three tadpoles from each of these three sites for both bacteria and viruses.
[b] Adequate histological sections were not obtained from one additional tadpole.
[c] Chytrid fungus (*Batrachochytrium dendrobatidis*) was cultured from four tadpoles and two recent metamorphs from this site.
[d] Histological results from the one tadpole were equivocal.
[e] Adequate histological sections were not obtained from two additional tadpoles.

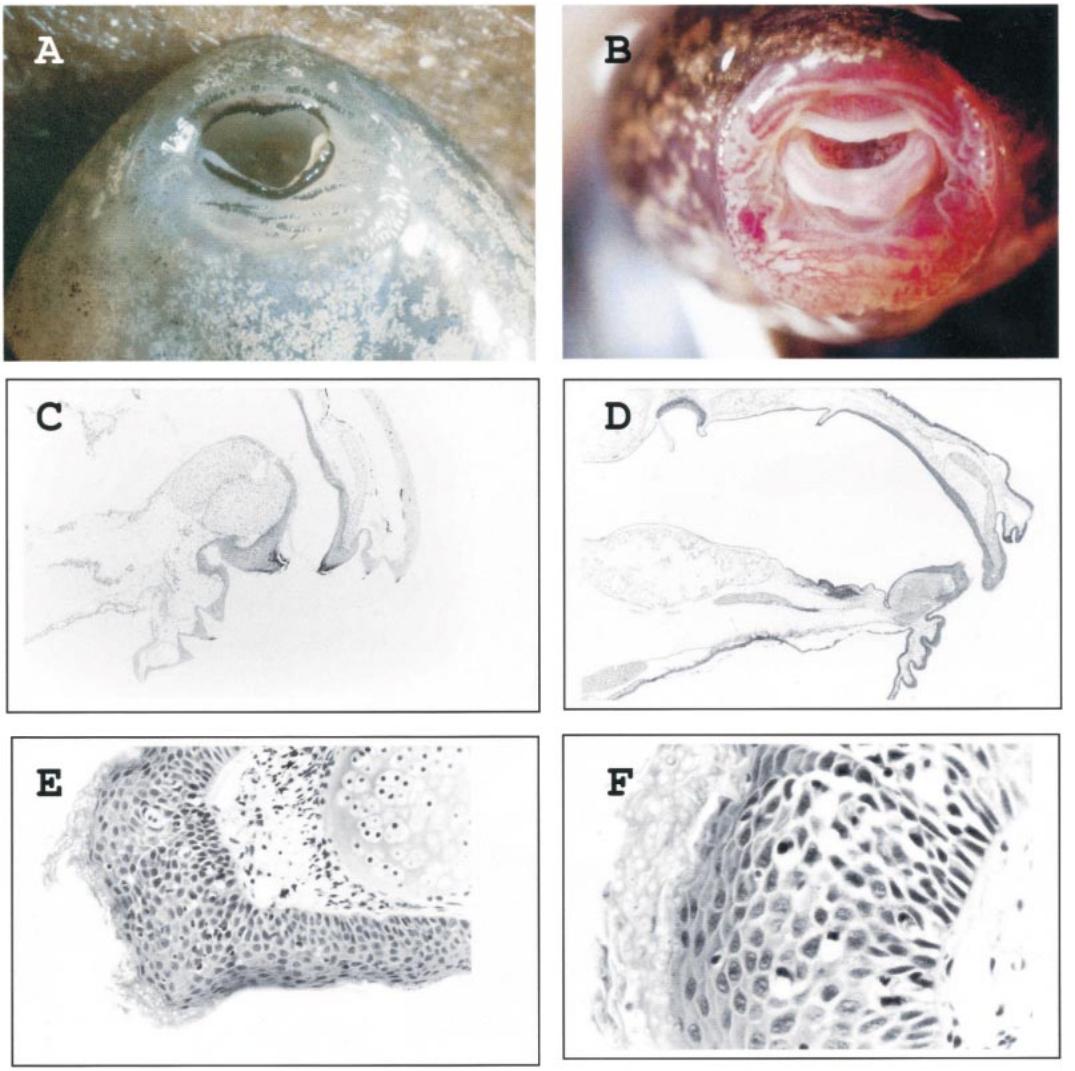

Fig. 1. Oral discs of a *Rana muscosa* tadpoles collected 25 1999 May at Y-764, Dry Creek, Mono County, California. (A) Note that there would normally be three toothrows on top and four on the bottom. In this individual, there are only short, broken, asymmetrical toothrows. The beak still retains most of the black pigment. The oral papillae do not show well, but they are neither swollen nor noticeably red. (B) All toothrows are missing in this individual; the beak is pale rather than jet black, and the oral papillae are swollen, and some of the papillae are noticeably red. (C) Normal oral disc in sagital section. Both jaw sheaths and two anterior and three posterior toothrows are present. Note the presence of black pigment at the tips of the jaw sheaths and toothrows and that the jaws and toothrows terminate in a point to various degrees. H&E stain. Accession number 98RaMu011. (D) Buccal cavity and oral disc in sagital section with oral chytridiomycosis. Note absence of black pigment at tips of jaw sheaths and toothrows, rounding of the tips of the jaw sheaths and toothrows, and increased thickness and cellularity in the epithelium of the same structures. H&E stain. Accession number 98RaMu141. (E) Posterior (lower) jaw sheath (same tadpole as panel C) showing marked oral chytridiomycosis. Note the complete absence of black pigment, the loss of a cutting edge, fraying of the surface epithelium, and the presence of numerous small clear spaces (chytrid zoosporangia) in the surface cells (see panel F). H&E stain. (F) Epithelium of posterior (lower) jaw sheath. Note the numerous round and ovoid clear chytrid zoosporangia in the pale thickened surface epithelium, absence of black pigment, and absence of an inflammatory cell reaction in the underlying dermis. H&E stain. Accession number 98RaMu143.

change in color (from jet-black to light brown) to a complete loss of all pigmentation.

Abnormal, asymmetrical toothrows were also found with little or no loss of pigment. Some tadpoles with normally pigmented (black) toothrows had abnormal toothrow formulae, misshaped toothrows, intersecting or fused toothrows, and missing toothrows (pigment and supporting basal ridge were absent, indicating the toothrow never formed and had not simply lost its pigmented labial teeth). All four tadpoles from Y-1579 had these types of abnormalities, even though their jaw sheaths were normal.

Histological findings.—Thalli of *Batrachochytrium* were observed histologically in keratinized epithelial cells of the jaw sheaths, toothrows, and palates of 16 of 24 tadpoles from five sites (66.7%, Table 1). In addition, one recent metamorph and one tadpole (that did not have an oral disc) from Y-764 had chytrid fungi on the toe tips. *Batrachochytrium* was not detected in the four tadpoles from Y-1579 and in one tadpole from Y-764, all of which had normally pigmented oral discs. Diagnostically useful sections of the jaw sheaths were not obtained on three tadpoles that had grossly abnormal jaw sheaths. *Batrachochytrium* thalli were present only within keratinized epithelial cells of the toothrows, jaw sheaths, and toe tips. No chytrids were found in the normally unkeratinized skin of the body, tail, eyes, limb buds, and labial papillae.

Depigmentation and loss of keratinized cells from the jaw sheaths and toothrows were the major histological features of chytrid-infected oral discs (Fig. 1C–D). The normally elongate black epithelial cells of the jaw sheaths and toothrows, which are oriented with their long axis perpendicular to the basement membrane, were absent. The pigmented cells were replaced by amelanotic (or very hypomelanotic) flat squamous epithelial cells, which were oriented with their long axis parallel to the basement membrane. These flattened epithelial cells had minimal to mild amounts of intracellular keratin and were the only cells infected by *Batrachochytrium* (Fig. 1E–F).

The fungal thalli in *R. muscosa* tadpoles had morphology typical of *B. dendrobatidis*. Zoosporangia (thalli) were spherical to ovoid; 3–11 microns in diameter; had a single discharge papilla that stained well in Giemsa, PAS, Steiner's silver, and Grocott's stains; had irregular filamentous rhizoids seen only in the Steiner's stain; and occasionally contained 1–2 micron diameter zoospores. The single discharge papilla of each fungal zoosporangium was consistently directed toward the cell surface, whereas rhizoids consistently were located on the opposite surface of the thallus (i.e., were directed toward the basement membrane). The majority of zoosporangia were empty, which indicates they had discharged their zoospores. Loss of pigmented epithelial cells in the jaw sheaths and toothrows was interpreted as erosion and metaplasia of these epithelial organs; ulceration was not evident. No concurrent infectious diseases were found in the oral discs.

Viral and bacterial culture results.—Viral cultures were negative in all nine tadpoles examined (Table 1, sites Y-321, Y-764, Y-1579), but eight species (six genera) of Gram-negative bacilli were isolated from tadpoles from these same sites. *Aeromonas hydrophila* was isolated from the mouths and intestines of three tadpoles from two sites; *Aeromonas veronii* biovar *veronii* was isolated from the mouth of one tadpole. *Comamonas acidovorans, Hafnia alvei, Pseudomonas fluorescens,* and *Spingomonas paucimobilis* were isolated from the mouths and intestines of four tadpoles from two sites. *Yersinia frederiksenii* was isolated from the intestinal samples. *Salmonella* and *Campylobacter* were not isolated from any specimens. No bacteria were isolated from the normally sterile internal organs (body cavity and liver).

Fungus culture results.—Four tadpoles, collected at site Y-764 on 17 October 1998 from under 2 cm of ice, had depigmented jaw sheaths and toothrows. One tadpole, however, was in metamorphic climax at Gosner stage 43–44 (Gosner, 1960) when lack of jaw sheaths and toothrows is normal. Attempts to isolate fungi from the depigmented jaw sheaths and the epidermis of two recent metamorphs and the late-stage tadpole resulted in isolates of *Batrachochytrium* from each animal. These isolates were morphologically indistinguishable from the type photographs of *Batrachochytrium dendrobatidis* (Longcore et al., 1999) and behaved the same in culture. The morphology and growth characteristics of *B. dendrobatidis* differ sufficiently from those of other genera of chytrids that confusion is unlikely.

Discussion

Oral and digital chytridiomycosis caused by a chytrid fungus (*B. dendrobatidis*) were found in *R. muscosa* tadpoles from five sites and in a recent metamorph (from one of the same sites) in or near the Sierra Nevada Mountains of California. All the infected tadpoles had conspicuous oral disc abnormalities including depigmented (white) toothrows, depigmented whitish jaw sheaths, and swollen, prominently vascularized

labial papillae. Equally important, all tadpoles with conspicuous oral disc abnormalities (and for which we had good histological preparations) had histologically confirmed chytrid infections.

No differences were detected in morphology among cultures of *Batrachochytrium* isolated from *R. muscosa* tadpoles, and from captive adult poison dart frogs (*Dendrobates azureus* and *Dendrobates auratus*) and White's treefrogs (*Litoria caerulea*) from the National Zoological Park in the United States (Longcore et al., 1999) and from captive *Limnodynastes dumerilli* from Australia.

No viruses were detected in cultures and histological examinations of the tadpoles. Because viral infections have caused die-offs of amphibians (Jancovich et al., 1997; D. Docherty, V. G. Chinchar, C. U. Meteyer, R. Brannian, W. Hansen, J. Wang, and J. Mao, 1999, unpubl.) and because some viral infections may cause temporary immune suppression resulting in secondary bacterial infections (Cunningham et al., 1996) or fungal infections, these negative viral findings are evidence that the chytrid fungal infections of tadpoles are not secondary to an underlying immunosuppressive viral epizootic. All bacteria that were isolated from the tadpoles are considered common environmental organisms or normal gut flora. Although several species of *Yersinia* may be important pathogens of humans, livestock, and wildlife, *Y. frederiksenii* is considered a common organism of soils and is nonpathogenic (Aleksic and Bockemuhl, 1999).

Oral disc abnormalities are not unique to infection by *Batrachochytrium*. Abnormalities of the toothrows, jaw sheaths, and anterior maxilla (snout) also have been reported in association with mine tailings, DDT exposure, and glucocorticosteroid exposure (Hayes et al., 1997). The histological features of the depigmented oral discs of tadpoles inhabiting surface water contaminated by mine tailings was not reported; hence, it is difficult to compare the lesions of this suspected intoxication to oral chytrid infection. Furthermore, it is uncertain whether a concurrent chytrid fungus infection contributed to the oral abnormalities associated with mine tailings. Senegal walking frog (Hyperolidae: *Kassina senegalensis*) tadpoles that were exposed to corticosterone had complete loss of the anterior jaw sheaths, anterior toothrows, and anterior labial papillae with fenestration of the anterior midline maxilla (Hayes et al., 1997). These abnormalities were analogous to cleft lip and cleft palate of mammals; no abnormalities of the posterior jaw sheaths and posterior toothrows were reported. Tadpoles in our study did not have the fenestrations or clefts associated with DDT and corticosterone intoxication, and the abnormalities of the oral discs involved the posterior jaw sheaths and posterior toothrows about equally. Hence, there are distinct differences between DDT intoxication and oral chytridiomycosis. Although it is possible that oral disc lesions without the maxillary midline fenestration (cleft) also may occur with DDT and corticosterone intoxication, this has not been reported. We conclude that oral chytridiomycosis alone was the cause of the abnormal (depigmented, eroded, and atrophied) oral discs in the *R. muscosa* tadpoles we studied.

Postmetamorphic amphibians can suffer fatal infections from this pathogenic chytrid (Berger et al., 1998; Pessier et al., 1999). It has been reported that recent metamorphs of the Australian *Mixophyes fasciolatus* suffered $> 90\%$ mortality within three weeks of completing metamorphosis. It is not known whether adult frogs acquire the fungus from tadpoles or whether the fungus is retained through the metamorphic process. Because larval *R. muscosa* typically overwinter and remain in the larval stage through 2–3 summers, chytrid-infected tadpoles may be transmitting *Batrachochytrium* to recent metamorphs and adult frogs. Also, although the adults of many frog species are present at the breeding sites for a short length of time in the spring, *R. muscosa* adults are present nearly all year long. Both these life-history traits may make *R. muscosa* particularly susceptible to chytrid fungal infections and may explain why this is one of the most seriously declining amphibians in the Sierra Nevada (Drost and Fellers, 1996; GMF, pers. obs.).

We cannot determine how long chytrid fungus has been present in the Sierra Nevada. Bradford (1991) reported a mass mortality of *R. muscosa* in the southern Sierra Nevada that he attributed to red-leg disease caused by *Aeromonas hydrophila* bacteria. Although chytrid fungus may have been present in this population, we have not observed the type of symptoms he reported (e.g., ventral surface of the thighs and sometimes the forearms and toes abnormally red with enlarged capillaries or hemorrhages). Our examinations of *R. muscosa* specimens in museums and our own collections indicate that infected tadpoles were present in 1993. Green and Kagarise Sherman (2001) reported chytrid in adult Yosemite toads (*Bufo canorus*) collected near Yosemite National Park as early as 1976. An examination of oral discs of tadpoles and the ventral skin of postmetamorphs in museums could help clarify the recent history and distribution of this pathogen in the Sierra Nevada.

Chytridiomycosis has only recently been found in salamanders (DEG, unpubl.). This may be be-

cause it is more difficult to detect in urodeles than anurans. Larval salamanders do not have keratinized toothrows or beaks that can be examined for abnormalities. In the absence of features that allow for efficient screening of potentially infected individuals, it is necessary either to conduct histological examinations of epidermal sections or to examine microscopically epidermis samples from salamander toes. It would be appropriate to examine Sierran populations of both long-toed salamanders (*Ambystoma macrodactylum*) and rough-skinned newts (*Taricha granulosa*) because they are both pond-breeding species that regularly occur within part of the range of *R. muscosa*.

Our research leads to two recommendations. First, field biologists should check tadpoles for oral disc abnormalities as an indication of chytrid fungal infections (and DDT intoxication). Because the fungus attacks the pigmented keratin of the toothrows and jaw sheaths, it is possible to look for signs of infection by examining the oral discs in the field with a 10× hand lens. Infected individuals typically will have toothrows and jaw sheaths that are mostly or entirely lacking black pigment; affected portions of the oral disc will appear whitish. Toothrows also tend to be notably bilaterally asymmetrical (Fig. 1A). Less commonly, tadpoles will have abnormally swollen and pinkish or reddened papillae (Fig. 1B). These types of abnormalities are a reliable indication of infection by the chytrid fungus *B. dendrobatidis* in tadpoles. Unfortunately, there are no readily observable features in larval or adult salamanders, or in adult frogs, that can be used to screen individuals for chytrid infection. Also, we have no data on whether tadpoles can be infected in the absence of mouthpart abnormalities, but it seems likely that this can occur, especially during early stages of infection.

Second, biologists must take precautions to prevent spreading *Batrachochytrium,* which reproduces asexually by means of minute, fragile, motile spores. If kept wet, free spores or spores within infected fragments of shed amphibian skin could be transported from one site to another on field equipment. Hence, field equipment should either be dried completely before being moved from one site to another, or equipment should be thoroughly washed and then rinsed in a disinfectant solution such as bleach (1 part bleach per 16 parts water).

Studies are needed to assess the effect of this fungal pathogen on the survivorship and recruitment of amphibians from breeding sites with and without chytrid-infected tadpoles. Persistence of population declines among several endemic amphibian species in the Sierra Nevada could be the result of very low survival rates and recruitment in recent metamorphs and to high mortality rates in adults that breed in ponds containing chytrid-infected second and third-year tadpoles. Furthermore, the prevalence and effect of chytrid infections in other species that have long-lived tadpoles (e.g., *R. catesbeiana, R. clamitans*) need to be assessed, because *Batrachochytrium*-infected bullfrog (*R. catesbeiana*) tadpoles have been found at other sites in California (GMF and DEG, unpubl.).

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APPENDIX 1

Locality codes, counties, elevation, latitude (dms), and longitude (dms) for sites where *Rana muscosa* larvae were collected.

L-553, Lassen, 1829 m, 40 15 11, 120 36 11; S-294, Fresno, 3370 m, 36 49 21, 118 25 58; S-309, Fresno, 3330 m, 36 49 17, 118 25 44; S-376, Tulare, 3420 m, 36 39 44, 118 25 14; S-387, Tulare, 3550 m, 36 40 47, 118 25 31; S-545, Fresno, 3300 m, 36 48 46, 118 25 35; S-562, Fresno, 3353 m, 36 48 09, 118 25 05; S-568, Fresno, 3425 m, 36 48 30, 118 24 44; T-083, Nevada, 2073 m, 39 20 06, 120 29 25; T-510, Mono, 2950 m, 38 10 23, 119 34 16; Y-029, Mariposa, 2225 m, 37 40 25, 119 39 07; Y-258, Madera, 2896 m, 37 37 29, 119 25 00; Y-321, Tuolumne, 3170m, 37 58 14, 119 20 42; Y-638, Tuolumne, 3294 m, 37 47 46, 119 13 38; Y-764, Mono, 2423 m, 37 52 58, 118 53 11; Y-764B, Mono, 2408 m, 37 53 00, 118 53 11; Y-765, Mono, 2408 m, 37 48 52, 118 50 37; Y-987, Mariposa, 3048 m, 37 51 32, 119 30 14; Y-1025, Tuolumne, 1658 m, 38 16 24, 120 08 58; Y-1219, Tuolumne, 3066 m, 37 50 36, 119 23 07; Y-1532, Mariposa, 2155 m, 37 41 32, 119 39 42; Y-1539, Mariposa, 2390 m, 37 36 46, 119 35 07; Y-1542, Mariposa, 2438 m, 37 36 40, 119 34 38; Y-1566, Madera, 2620 m, 37 36 32, 119 29 51; Y-1579, Madera, 2587 m, 37 36 46, 119 30 43; Y-1608, Madera, 2685 m, 37 37 35, 119 26 58; Y-1610, Madera, 2728 m, 37 37 28, 119 26 32; Y-1665, Mariposa, 2981 m, 37 42 40, 119 26 03; Y-1849, Madera, 3018 m, 37 39 29, 119 22 58; EWA-1, Tuolumne, 2737 m, 38 09 44, 119 41 53; EWA-2, Tuolumne, 2805 m, 38 10 05, 119 41 55; MVZ-2, Fresno, 2306 m, 36 44 52, 118 45 10; MVZ-3, Nevada, 1920 m, 39 26 10, 120 13 54; MVZ-4, Mariposa, 2355 m, 37 42 23, 119 35 24; MVZ-5, Alpine, 2628 m, 38 29 24, 119 48 23.