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# First Report of Chytridiomycosis (*Batrachochytrium dendrobatidis*) in Endangered *Neurergus microspilotus* (Caudata: Salamandridae) in Western Iran

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**Abstract:** We were able to distinguish immature and mature zoosporangia, zoospores and discharge tubes of chytrid fungus, *Batrachochytrium dendrobatidis*, in live and dead endangered yellow spotted mountain newt (*N. microspilotus*) in Kavat Stream in western Iran. Histological evidences provided from finger toes confirm the presence of *B. dendrobatidis* in the host keratinized epithelial cells of the skin tissue. The newts diagnosed with chytridiomycosis in our study included two dead adult *N. microspilotus* collected from the Kavat Stream and two juveniles kept in laboratory. Clinical symptoms in dead *N. microspilotus* kept in a laboratory included lack of appetite, loss of weight, loss of digits, accumulation of gelatinous material on the skin and plain fungal infections on the body. This study provides the first record of the presence of *B. dendrobatidis* in live and also cases of chytridiomycosis in dead yellow spotted mountain newts from Kavat Stream in western Iran.

**Key words:** *Batrachochytrium dendrobatidis* · *Neurergus microspilotus* · Histopathology · Kavat Stream

## INTRODUCTION

According to the evaluation made by IUCN on the conservation status of world amphibians this group of vertebrates are among the most threaten taxa in the world [1]. Population decline caused by various factors such as habitat loss, exotic species, UV radiation, acid precipitation, climate change, environmental pollution and infectious disease have brought over one-third of amphibian species to the verge of extinction [2, 3]. The causes of the global decline in the amphibian populations are a matter of continued research, but infectious diseases are increasingly recognized as key threats to animal populations [4, 5]. Reports of chytridiomycosis as a serious disease of amphibians caused by the fungus *B. dendrobatidis* shows that this disease occurs in many localities in Africa [6], Australia [7], Europe [8], North America [9], Asia [10], Central America [11] and South America [12]. The infection has been found in both wild and captive populations [13]. *B. dendrobatidis* is an important chytrid fungus able to develop chytridiomycosis in most of the amphibian species. Many of these species are linked to shocking population declines and even species extinctions [14]. Declines in amphibian population as a result of chytridiomycosis can occur very rapidly sometimes over few weeks [15]. This disease is able to disproportionately eliminate species that are rare, specialized and endemic [16].

*Batrachochytrium dendrobatidis* (*Bd*) appears to be specific to amphibians [7] and is one of only two species of chytrid fungus known to parasitize vertebrates [17]. *Bd* infection has been documented in numerous frog species, some salamander species and a single caecilian species (*Typhlonectes* sp.) in captivity [18]. The origin of *Bd* is still unknown [14] and hypotheses regarding possible sources, including Africa, Asia, or North America, are currently being debated [10]. Meanwhile, amphibian declines attributed to *Bd* continue to be reported in various regions including North America [19], Central America and Asia [20]. As a result of its global impact, the World Organization for Animal Health

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(OIE) recently listed *Bd* as a notifiable pathogen [21], the first to be included for its threat to biodiversity. Although there have been no reports of chytridiomycosis transmission to other aquatic vertebrates the potential spread of infectious diseases between amphibians and fishes have been inferred [22].

Chytridiomycosis can only be diagnosed based on direct examination of skin scrapings [23]. These examinations could be based on histological evidence [24], immunohistochemistry, electron microscopy [25], standard polymerase chain reaction (PCR) [26], real time PCR [13], nested PCR [10] and by culturing. Most of these techniques have a high positive predictive value when used on heavily infected amphibians. However, chytridiomycosis is a common disease and can also be found in healthy individuals. Real-time PCR is a highly sensitive technique and can detect *B. dendrobatidis* within a short time after infection. At present, real-time PCR appears likely to become the test of choice for lightly infected or healthy amphibians [27]. The real-time PCR test does not involve removal of a toe or scarifying the amphibian and can be done on frozen or ethanol-stored tissues, skin swabs, or even in a weak saline solution in which the amphibian has been submerged [28].

There are at least 21 amphibian species reported from Iran [29] including some that are critically endangered by IUCN criteria. The Salamandridae are represented by three genera, *Triturus*, *Neurergus* and *Salamandra* [29]. The genus *Neurergus* has a relatively wide geographic distribution, ranging from southern Zagros Mountains to mid-Zagros range and extending into Iraq and southern Turkey. The yellow spotted mountain newt occurs in Iran and Iraq and listed as critically endangered by IUCN. This species occurs in different climatic regimes including warm and dry areas in eastern Iraq and also cold and wet areas at higher elevation in mid-Zagros Range in Iran [30]. Presence of *B. dendrobatidis* or development of chytridiomycosis has not been reported in any species of amphibian in Iran. In this study we report cases of the chytrid fungus and development of chytridiomycosis in *N. microspilotus* in Kavat Stream in western Iran.

## MATERIALS AND METHODS

*N. microspilotus* samples included in this study were restricted to 10 adult toe clips from Kavat Stream (N 34°52.687' E 46°36.433') collected in June 2011. Post-metamorphic and adult yellow spotted newts were collected by hand in the day. The newts were examined for any clinical symptoms associated with chytridiomycosis. The longest finger of the forelimb (i.e. second or third) was cut and the wound disinfected with Betadine. Samples were preserved in 10% formalin solution in separate vials. Finally, specimens were released in the streams.

The phalanges were decalcified in a solution of ethylene diamine tetra acetic acid (EDTA) for 5-7 days. Toe samples were dehydrated in a graded alcohol series, cleared in xylene and embedded in wax. Microscopic sections were prepared from toe clips through generating serial sections with a thickness of 6 microns. Then, tissues were stained using Hematoxylin and Eosin (HE). The sections were then examined and photographed under a camera microscope (Leica with Dinocapture 2.0), allowing for simultaneous comparison and facilitating the analysis of chytrid infections. Chytrid infections were diagnosed based on the presence of zoosporangia with discharge papilla embedded in the skin epithelium [26]. Each section was individually examined and scored as infected or not.

## RESULTS

Clinical symptoms in two infected adult *N. microspilotus* kept in a captive breeding facility at Razi University included lethargy, lack of appetite, sitting unprotected during the day, reduced or loss of righting reflex, skin lesions, sloughing or peeling on the outside layers of skin, erosions and ulcerations (Fig. 1). These individuals in last stages of the infection before death were covered by plain fungus (Fig. 1). These specimens were subjected to histological examination for chytridiomycosis (Fig. 2). In the toe clip collected from live newts in Kavat Steam, there was a moderate to marked hyperplasia of the epidermis with some hyperkeratosis and occasionally mphocytic or granulocytic infiltrates in the dermis and, less frequently, the epidermis. In the hyperplastic epidermis, there were few to many intracellular fungal

Fig. 1: A dead *N. microspilotus* kept in a captive breeding facility at Razi University tested positive to *B. dendrobatidis*.

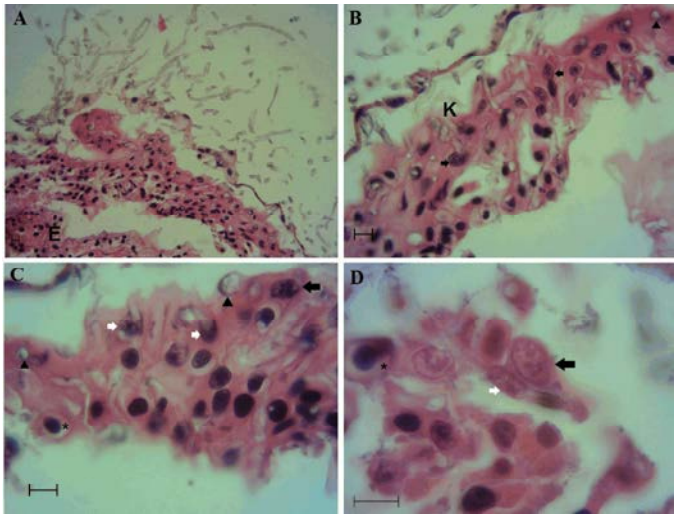


Fig. 2: A section of skin from an infected adult *N. microspilotus*. Note immature stage (asterisks). Mature zoosporangium with discrete basophilic Zoospores (black arrows) and discharge tube (with arrows) containing zoospores and empty zoosporangium after zoospores have discharged (arrows heads). E = epidermis, K= keratin layers. Scale: largest zoosporangia are 15ìm in diameter. H and E. Bar = 10 µm.

organisms (Fig. 2 A and B). The fungi consisted of sporangia, 10-15 µm in diameter, which often contained 2-4 small zoospores, approximately 2-3 µm in diameter (Fig. 2C). Large number sporangia, the flask shape and discharging tube, were clearly visible. Zoospores can be released via the discharge papilla. On some stained sections the rhizoids are present (Fig. 2D).

## DISCUSSION

This study provides the first record of the presence of *B. dendrobatidis* in live and dead tissues of *N. microspilotus*. This study also provides cases of chytridiomycosis in free living and captive yellow spotted mountain newt from Kavat Stream in western Iran. As shown in other species of amphibians, *B. dendrobatidis* observed in *N. microspilotus* are limited entirely to keratinized epithelial cells of the skin in adult or postmetamorph individuals. Severe infections by *B. dendrobatidis* in *N. microspilotus* are limited to the stratum corneum of the epidermis and consist of scattered sporangia or small clusters of the organisms within the cytoplasm of host cells (Fig. 2D). *N. microspilotus* occurs in Iran and Iraq and listed as critically endangered by IUCN. This newt has been reported to occur in 14 first order streams in southern Zagros Mountains normally in very low number of individuals. It has also been reported in several localities in eastern Iraq. The possible role of chytridiomycosis in distribution and abundance of this species is not known and further investigations are required to resolve this inquiry clarify.

Most studies use histopathological analysis or real-time PCR to determine the presence or absence of *Bd* at the level of individuals in a population. Such studies are less informative than studies examining prevalence within populations and communities over time. It is also possible to focus on the ways the chytrid disease can be transferred between different species at community level. Some authors believe it is time to shift our attention to the question of population and community-level effects of this disease. Diagnosis of chytridiomycosis based on direct histological examination of skin tissue is not adequately sensitive and can have a high positive predictive value when used on heavy infected amphibians. However, chytridiomycosis is a common disease and can also be found in healthy individuals. Further testing is needed for additional species of amphibians throughout the country to assess potential threats.

In subacute and advanced infections, massive numbers of spherical to slightly ovoid intracytoplasmic thalli will be found in multiple retained layers of unshed epidermis (hyperkeratosis). Acanthosis also may be present and the number of cell layers in the epidermis may increase greatly from a normal number (3-5) to a much larger number (= 8-15) [30]. Most thalli will appear to be empty (clear), but some will contain indistinct basophilic material in the core, while a few others will contain multiple distinct minute basophilic roughly spherical zoospores [31]. Similar to other amphibians, *N. microspilotus* that die of lethal chytridiomycosis are likely to show epidermal changes that are easily detected

and have large numbers of *B. dendrobatidis* zoosporangia infecting their epidermis [32]. Clinical symptoms in living and dead *N. microspilotus* tested positive to chytridiomycosis include lethargy, lack of appetite, loss of digits, irregular skin sloughing, weight loss, accumulation of gelatinous material and fungal infections on the body.

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## REFERENCES

1. Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman and R.W. Waller, 2004. Status and trends of amphibian declines and extinctions worldwide. Science, 306: 1783-1786.
2. Wake, D.B. and V.T. Vredenburg, 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proc. Natl. Acad. Sci. USA., 105: 11466-11473.
3. Hof, C., M.B. Araújo, W. Jetz and C. Rahbek, 2011. Additive threats from pathogens, climate and land-use change for global amphibian diversity. Nature, 480: 516-519.
4. Carey, C., D.F. Bradford, J.L. Brunner, J.P. Collins, E.W. Davidson, J.E. Longcore, M. Ouellet, A.P. Pessier and D.M. Schock, 2003. Biotic factors in amphibian population declines, pp: 153-208 in G. Linder, S.K. Krest and D.W. Sparling, editors. Amphibian decline: an integrated analysis of multiple stressor effects. Soc. Environ. Tox. Chem. Pensacola, Florida.
5. Collins, J.P. and A. Storfer, 2003. Global amphibian declines: sorting the hypotheses. Diver. Distrib., 9: 89-98.
6. Lane, E.P., C. Weldon and J. Bingham, 2003. Histological evidence of Chytridiomycosis in a free-ranging amphibian *Afrana fuscigula* (Anura: ranidae) in South Africa. J. S. Afr. Vet. Assoc., 74: 20-1.
7. Berger, L., R. Speare, P. Daszak, D.E. Green, A.A. Cunningham, C.L. Goggins, R. Slocombe, M.A. Ragan, A.D. Hyatt, K.R. McDonald, H.B. Hines, K.R. Lips, G. Marantelli and H. Parkes, 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc. Nat. Acad. Sci. USA., 95: 9031-9036.
8. Bosch, J., I. Martinez-Solano and M. Garcia-Paris, 2001. Evidence of chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of Central Spain. Biol. Conserv., 97: 331-7.
9. Daszak, P., A.A. Cunningham and A.D. Hyatt, 2003. Infectious disease and amphibian population declines. Divers. Distrib., 9: 141-50.
10. Goka, K., J. Yokoyama, Y. Une, T. Kuroki, K. Suzuki, M. Nakahara, A. Kobayashi, S. Inaba, T. Mizutani and A.D. Hyatt, 2009. Amphibian chytridiomycosis in Japan: distributions, haplotypes and possible route of entry into Japan. Mol. Ecol., 18: 4757-4774.
11. Lips, K.R., 1999. Mass mortality and population declines of anurans at upland site in western Panama. Conserv. Biol., 13: 117-25.
12. Herrera, R.A., M.M. Steciow and G.S. Natale, 2005. Chytrid fungus parasiting the wild amphibian *Leptodactylus ocellatus* (Anura: leptodactylidae) in Argentina. Dis. Aquatic. Organ., 64: 247-252.
13. Boyle, D.G., D.B. Boyle, V. Olsen, J.A.T. Morgan and A.D. Hyatt, 2004. Rapid quantitative detection of Chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis. Aquat. Org., 60: 141-148.
14. Fisher, M.C., 2009. Endemic and introduced haplotypes of *Batrachochytrium dendrobatidis* in Japanese amphibians: sink or source? Mol. Ecol., 18: 4731-4733.
15. Lips, K.R., F. Brem, R. Brenes, J.D. Reeve, R.A. Alford, J. Voyles, C. Carey, L. Livo, A.P. Pessier and J.P. Collins, 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc. Nat. Acad. Sci. USA., 103: 3165-3170.
16. Smith, K.G., K.R. Lips and J.M. Chase, 2009. Selecting for extinction: nonrandom disease-associated extinction homogenizes amphibian biotas. Ecol. Let., 12: 1069-1078.

17. Èervinka, S., J. Vtovec, J. Lom, J. Hoka and F. Kubù, 1974. Dermocystidiosis a gill diseases of the carp due to *Dermocystidium cyprini*. J. Fish. Biol., 6: 689-699.
18. Raphael, B. and J. Pramuk, 2007. Treatment of chytrid infection in *Typhlonectes* spp. using elevated water temperatures. Proc. IRCEB meeting, Phoenix, Arizona, Unpublished.
19. Davidson, E.W., M. Parris, J.P. Colline, J.E. Longcore, P. Pessier and J. Brunner, 2003. Pathogenicity and transmission of Chytridiomycosis in Tiger Salamanders (*Ambystoma tigrinum*). Copeia, 3: 601-607.
20. Puschendor, R., F. Bolanos and G. Chaves, 2006. The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. Biol. Conserv., 132: 136-142.
21. Schloegel, L.M., C.M. Ferreira, T.Y. James, M. Hipolito, J.E. Longcore, A.D. Hyatt, M. Yasbley, A.M.R.P.F. Martins, R. Mazzoni, A.J. Davies and P. Daszak, 2010. The North American bullfrog as a reservoir for the spread of *Batrachochytrium dendrobatidis* in Brazil. Anim. Conserv., 13: 53-61.
22. Green, E.D. and K.J. Dodd, 2007. Presence of amphibian chytrid fungus *Batrachochytrium dendrobatidis* and other amphibian pathogens at warm water fish hatcheries in southeastern North America. Herpetol. Conserv. Biol., 2(1): 43-47.
23. Briggs, C. and S. Burgin, 2004. Congo red, an effective stain for revealing the chytrid fungus, *Batrachochytrium dendrobatidis*, in epidermal skin scrapings from frogs. Mycologist., 18: 3.
24. Pessier, A.P., D.K. Nichols, J.E. Longcore and M.S. Fuller, 1999. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). J. Vet. Diagn. Invest., 11: 194-199.
25. Berger, L., A.D. Hyatt, V. Olsen, S.G. Hengstber, D. Boyle, G. Marantelli, K. Humpreys and J. Longocore, 2002. Production of polyclonal antibodies to *Batrachochytrium dendrobatidis* and their use in an immunoperoxidase test for chytridiomycosis in amphibians. Dis. Aquat. Organ., 48: 213-220.
26. Annis, S.L., F.P. Dastoor, H. Ziel, P. Daszak and J.E. Longcore, 2004. A DNA based assay identifies *Batrachochytrium dendrobatidis* in amphibians. J. Wildl. Dis., 40: 420-428.
27. Boyle, D.G., D.B. Boyle, V. Olsen, J.A.T. Morgan and A.D. Hyatt, 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real time Taqman PCR assay. Dis. Aquat. Organ., 60: 141-148.
28. Brem, F., J.R., I.I.I. Mendelson and R.K. Lips, 2007. Field-Sampling Protocol for *Batrachochytrium dendrobatidis* from Living Amphibians, using Alcohol Preserved Swabs. Version 1.0 (18 July 2007). Electronic document accessible at http://www. amphibians.org Conservation International, Arlington, Virginia, USA.
29. Baloutch, M. and H.G. Kami, 1995. Amphibians of Iran. Tehran University Publications, 177: 91-99.
30. Sharifi, M. and S. Assadian, 2002. Distribution and conservation of *Neurergus microspilotus*. Asi. Herpetol. Res., 10: 224-229.
31. Densmore, C.L. and D.E. Green, 2007. Diseases of amphibians. I.L.A.R. J., 48: 235-254.
32. Kriger, K.M. and J.M. Hero, 2007. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. J. Zool., 271: 352-359.