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Dermocystid infection and associated skin lesions in free-living palmate newts (*Lissotriton helveticus*) from Southern France

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ABSTRACT

Since the early 1900s, mesomycetozoan parasites have been reported in both European anuran and caudate species. These reports have primarily been descriptive, which has made assessing the impact of these parasites on host populations difficult. Anecdotal reports of dermocystidium-like parasites are becoming widespread across Europe, possibly indicating that these mesomycetozoan parasites are increasing in distribution and/or abundance. This highlights the need for further investigations into the occurrence, pathogenesis and effects on host health of these parasitic infections for free-living amphibian populations, particularly those which are already stressed or threatened by other factors. Here we report the results of pathological, microbiological and molecular investigations used to characterize unidentified skin lesions in palmate newts (*Lissotriton helveticus*) from Larzac, France. We confirm that the lesions are the result of infection with a novel dermocystidium-like parasite, which is related to *Amphybiocystidium ranae*. We also show that the same parasite is distributed across several newt breeding sites. The lesions that result from infection with this parasite range from single or few vesicular or nodular cutaneous lesions to multiple coalescing skin ulcers with extensive hemorrhages. The latter have not been previously described in amphibians due to mesomycetozoan parasitic infection. Dermocystid DNA was detected only in newts that showed lesions, providing comparative evidence of the parasite's pathogenicity. We discuss the potential significance of the presence of this pathogen in the context of the population health of palmate newts.

1. Introduction

Dermocystidians form one of two branches of the class Mesomycetozoea, a protozoan lineage often cited as the ancestor to all metazoans [1,2]. While the true relationships between the Fungi, Animalia and Mesomycetozoea remain unresolved, it is clear that mesomycetozoans, along with the choanoflagellates, occur somewhere at the boundary between animals and fungi [3,4]. Members of the order Dermocystida infect hosts from nearly all the vertebrate orders and several genera utilize amphibian hosts, in some cases exclusively [3,5,6]. Gross signs of infection of amphibian hosts can appear severe (Fig. 1), yet the pathogenesis and impact of dermocystidian parasites in amphibian hosts remain poorly studied. While Pascolini et al. [6] described a dermocystidian affecting an apparently declining amphibian population; they avoided invoking cause and effect in their descriptive study [7]. Raffel et al. [8] reported an increased mortality of red-spotted newts (*Notophthalmus viridescens*) visibly infected with *Amphibiocystidium viridescens*, however they, too, avoided making a case for cause and effect due to the correlative nature of their data. Green et al. [9] provided further correlative evidence that cannot unambiguously attribute the cause of mortality or morbidity to a dermocystidian parasite. Nevertheless, all these studies make strong cases for dermocystidian parasites causing clinical signs of disease and leading to amphibian mortality, mirroring that seen in fish hosts lethally affected by dermocystidian infections [3,10].

Since the beginning of the twentieth century, dermocystid mesomycetozoan parasites have been reported in a number of European amphibian species (e.g. marbled newts (*Triturus marmoratus*) [11,12]; common frogs (*Rana temporaria*) [13,14] and *Pelophylax esculentus* complex [6,15]). Most reports of infections in Europe are primarily descriptive, lacking robust and comparative sampling efforts, unlike efforts made by researchers outside the European

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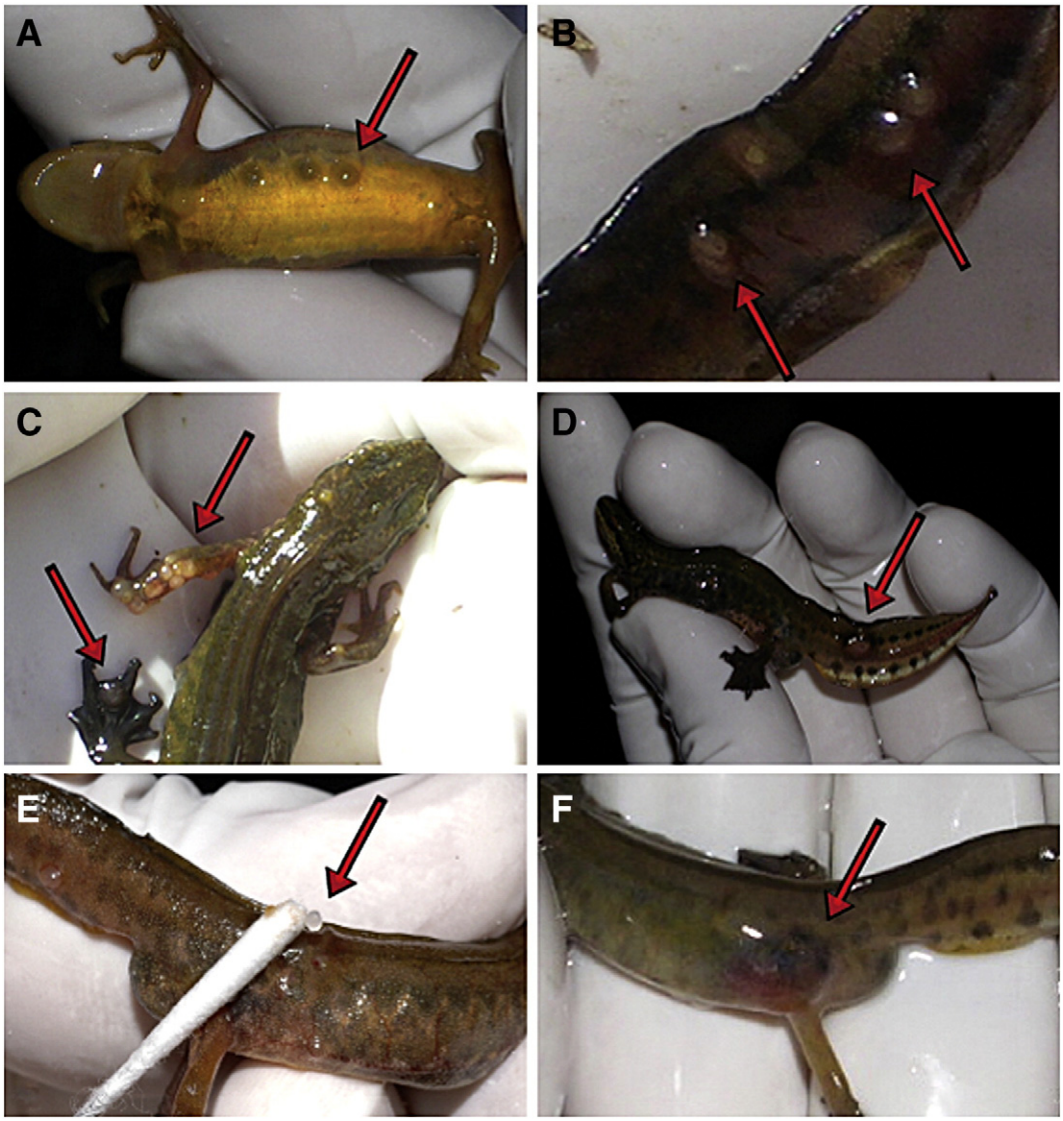

Fig. 1. Gross skin lesions in the palmate newt. A) Vesicles, B) carbuncle-like preulcerative lesion, C) multifocal preulcerative lesions, D) cluster of preulcerative lesions, E) ulcer and F) spherical white cyst detached from a preulcerative lesion.

continent (e.g. [8,16,17], but see [6]). This is unfortunate, as amphibian parasites, specifically ranavirus and *Batrachochytrium dendrobatidis*, have been linked to mass mortality events and declines of European amphibians [18–23]. Extensive and/or robust sampling for *B. dendrobatidis* has shown that this lethal amphibian parasite has not reached equilibrium in Europe, and continues to extend its range in both space and host [24]. A similar situation is thought to occur for ranavirus infection, at least in the United Kingdom [18], but little research has been conducted on this pathogen in amphibians in Europe [see 25]. Anecdotal reports of dermocystidian-like infections of European amphibians appear to be wide-ranging. Such observations may indicate dermocystidian parasites are extending their ranges in Europe and highlight the need for further investigations into the occurrence, pathogenesis and health significance of mesomycetozoan parasites in free-living amphibian populations. In particular, dermocystid parasites might present a threat to the survival of amphibian populations that are already stressed or threatened by other factors [26,27].

Recently a high frequency of vesicular skin lesions in palmate newt (*Lissotriton helveticus*) in populations in Southern France was observed (M. Denoël, pers. obs.). Superficially, these lesions appeared similar to those generated by dermocystidian infections of other amphibian hosts. However, the etiology of these lesions and their health significance to palmate newts has not yet been established. Here, we report the results of pathological, microbiological and molecular investigations used to characterize these skin lesions, identify the causal and associated agents, confirm infection with a dermocystidian parasite using a comparative framework, show infection occurs at multiple newt breeding locations and discuss the potential significance to the palmate newt population's health.

2. Materials and methods

2.1. Study site

The study was conducted at the Larzac limestone plateau, located in the south of France (Department of Herault, Languedoc-Roussillon), where there are numerous temporal ponds inhabited by the native palmate newt (*L. helveticus*), and two other newt species [alpine newts (*Messotriton alpestris*) and marbled newts (*T. marmoratus*)], in addition to several species of anurans [28–30]. On the basis of previous observations (M. Denoël, unpubl. data), three ponds were selected as sampling sites: Pond "A" Bagnelades (1 × 1 km UTM coordinates: 0529–4855), Pond "B" Pioch de Labro (0523–4859) and Pond "C" Les Labres (0530–4856).