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First steps to survey chytrid fungus in Croatia

Prvi koraci u istraživanju chytrid gljivica u Hrvatskoj

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The *Batrachochytrium dendrobatidis* (Bd) (LONGCORE ET AL., 1999) is a recently emerged amphibian pathogen, causing the disease chytridiomycosis, which has been identified as a cause of mass amphibian mortalities and a factor influencing world-wide amphibian declines (STUART ET AL., 2004, POUNDS ET AL., 2006). The disease has been considered responsible for amphibian declines in the Americas, Caribbean, Africa, Australia, New Zealand, and the range of the pathogen recently expanded into Europe (GARNER ET AL. 2005). Within Europe the infection was detected already in about 1/3 of Europe's amphibian fauna in 12 countries (GARNER ET AL. 2005, VÖRÖS ET AL. 2009, SURA ET AL. 2010, SZTATECSNY & GLASER, 2011, SPATIELEPIDEMIOLOGY, 2011).

The Easternmost occurrence of the pathogen in Europe is proved from the low elevation mountains of Hungary, where no Bd-linked mortalities have been reported yet (VÖRÖS ET AL., 2009). South-eastern Europe and the Balkan Peninsula seem to be mostly unexplored regarding the distribution of the fungus. GARNER ET AL. (2005) sampled 8 individuals of *Rana latastei* from low elevation area of coastal Croatia, which tested negative for Bd (GARNER, pers. comm. 2005). Since Croatia has high elevation mountains and climatic conditions in

these regions could be suitable for Bd, a systematic survey is needed to infer the presence/absence of the pathogen.

In 2010 we started sampling of Croatian amphibians on three species (*Bombina variegata* (11), *Ichthyosaura alpestris* (1), *Pelophylax esculentus* (1)) and on two locations close to Zagreb (Veliki Potok, Mikulići), in order to explore the possible Bd infection in the area. It is well documented that introduced frog species can be vectors of chytridiomycosis and also release of exotic species can be responsible for spread of the disease (GARNER ET AL., 2007, SOTO-AZAT ET AL., 2010). For this reason we choose to start sampling mountainous habitats near the capital.

The specimens were sampled on their back, groin and webbing area with a sterile cotton swab (Biolab). We used the standard real-time PCR protocol (BOYLE ET AL., 2004) to detect Bd. Tests were run in the Laboratory for Molecular Taxonomy of the Hungarian Natural History Museum, Budapest, using an ABI Step One real-time PCR machine.

All the 13 individuals tested negative for chytrid fungus. This is a very low sampling size and no significant consequences can be drawn from the results, but practice in Hungary shows that *Bombina variegata* is a good indicator for presence of Bd (VÖRÖS ET AL., 2009). We plan more studies focusing on high elevation areas and sampling *Bombina variegata* in order to further assess the distribution of chytrid fungus within South-eastern Europe.

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