Herpetology Notes 19: 269–272, published on 16 May 2026

Pseudomonas aeruginosa skin infection after long-term captivity of a Dalmatian Wall Lizard, *Podarcis melisellensis* (Braun, 1877)

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The Dalmatian Wall Lizard, *Podarcis melisellensis* (Braun, 1877), is a medium-sized endemic lacertid (adult snout-vent length up to 70 mm) that inhabits a variety of arid habitats along the eastern Adriatic coast and on islands in the Adriatic Sea (Arnold and Ovenden, 2002; Brecko et al., 2008; Ajtic et al., 2009). This species is found in dry rocky hills, meadows and various anthropogenic landscapes (olive grows, orchards, vineyards, pastures) but evades urban areas (Grbac and Bauwens, 2001; Huyghe et al., 2007).

In veterinary practise, lizards with skin diseases are frequently encountered in captivity. These diseases are primarily the result of environmental stress due to improper husbandry and feeding as well as an underlying disease that leads to secondary bacterial or fungal skin infections (Hellebuyck et al., 2012). Several pathogens can act as primary etiological agents of dermatitis and septicaemia and cause high morbidity (and even mortality) in lizards, or only localised infection of a specific part of the skin (Hellebuyck et al., 2012; Supic et al., 2021; Xiong et al., 2022). In particular, high humidity, low or excessively high temperatures, poor nutrition or damaged skin can be potential factors for the development of bacterial infections (Palmeiro and Roberts, 2013). Gram-negative bacteria, which are normally found in the environment, are the most common causes of bacterial skin infections in lizards, including bacteria of the genus *Pseudomonas* (Hoppmann and Barron, 2007). *Pseudomonas aeruginosa* is a ubiquitous pathogen (gram-negative, rod-shaped and aerobic bacterium) that is widely distributed in the environment, and occurs as an opportunistic pathogen in many animals, including lizards (Grosso-Becerra et al., 2014; Hattab et al., 2021). These bacteria can cause infection of the skin, respiratory tract and digestive system (and septicaemia) in reptiles (Seixas et al., 2014; Xiong et al., 2022). According to current knowledge, there is no information on diseases of *P. melisellensis*.

A subadult male of the Dalmatian Wall Lizard, *P. melisellensis*, was caught with a noose in vicinity of Sinj (43.7029°N, 16.6375°E), central part of Eastern Adriatic coast, Croatia, in September 2021. The animal was collected as part of a scientific project with provided permits for collecting wild animals. It was transferred to the Department of Biology, of the Faculty of Sciences, University of Zagreb and kept in a plastic terrarium 38 x 28 x 25 cm (wide x deep x high). After a year, as the experimental part of project ended, it was moved to a glass terrarium measuring 60 x 30 x 30 cm (wide x deep x high), which was designed as naturally as possible: water bowl, natural hiding place, cork bark, and stones. The animal still lives in this terrarium (August 2025). The terrarium is equipped with UVB lamp and a radiant heat lamp. In August 2024, an approx. 3 mm scab was found on both sides of the lizard's cheek (Fig. 1).

After removing the scab from the deeper bilateral symmetrical longitudinal lesions in the cheek area, which were approximately 3 mm long and 1 mm wide and deep, swab samples were taken for standard microbiological examination (bacteriological and mycological) using a sterile culture swab. Also, three impression smears on a glass slides were made for a cytological examination at the Hematological laboratory at the Department of Pathophysiology of the Faculty of Veterinary Medicine, University of Zagreb, Croatia. Impression smears were obtained by gently pressing the

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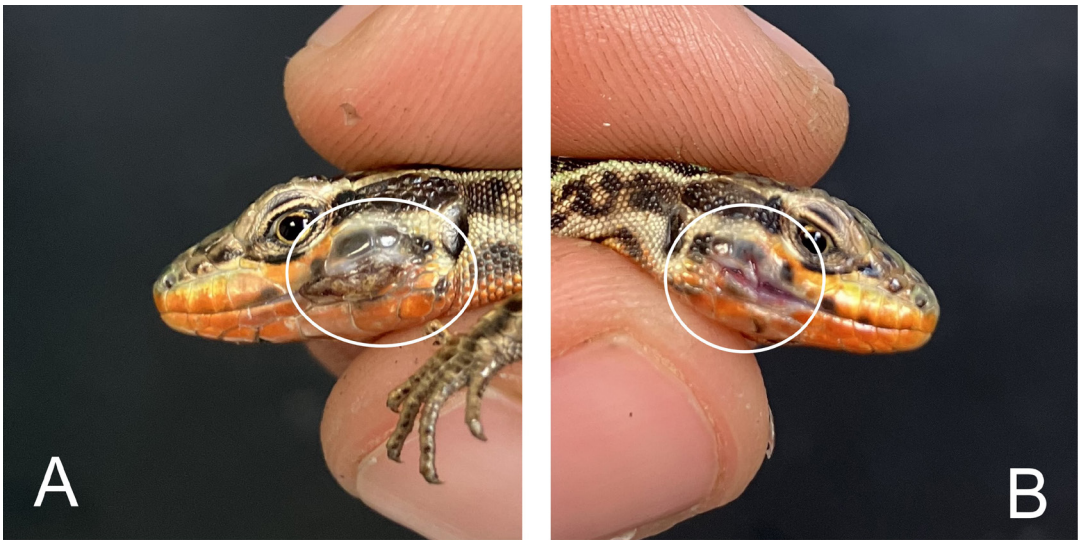

Figure 1. Male of the Dalmatian wall lizard, *P. melisellensis*, (A) with scab on the cheek region on the left side of the head marked with white circle, and (B) after removing scab on the cheek region on the right side of the head marked with white circle. Photos by Josip Miljković.

lesion directly onto clean glass slides, without the use of any additional instruments. The affected areas were disinfected with cotton wool soaked in povidone-iodine (Betadine 10% solution, Alkaloid d.o.o., Ljubljana, Slovenia).

The microbiological agents and the antibiogram were determined according to the standard microbiological and CLSI protocols (CLSI, 2024) in the Bacteriological laboratory of the Department of Poultry Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, Croatia. The three glass slides with the impression smears were air-dried, stained with May-Grünwald Giemsa staining procedure and dried again before microscopic examination under a 100x objective (Nikon E200, Tokyo, Japan).

The isolated bacteria (*P. aeruginosa*) were resistant to most antibiotics (penicillins, cephalosporins, tetracyclines and fluorphenicols) and sensitive only to quinolones (enrofloxacin (+++), marbofloxacin (+++)) and sulphonamides with trimethoprim (++). The infection was confirmed by cytological findings as well. Focally, a round cell was observed containing a small amount of fine, intracytoplasmic, dark blue to black granular material (melanophore) (Fig. 2A). The cytological background additionally contained a small amount of discrete to aggregated round cells, heterophils, monocytes, few reactive lymphocytes, many red blood cells, and a large amount of dark blue rod-shaped bacteria that were present both intracellularly in heterophils and monocytes as well as extracellularly (Fig. 2B). Multidrug-resistant strains of *P. aeruginosa* are very frequently isolated from various animal species and humans and pose a significant health problem (Grosso-Becerra et al., 2014; Hattab et al., 2021; Cristina et al., 2022). The *P. aeruginosa* bacteria isolated from reptiles are resistant to most antibiotics (penicillins, cephalosporins, macrolides, lincosamides, tetracyclines, sulfonamides + trimethoprim, chloramphenicol and florphenicols) and sensitive only to quinolones and some aminoglycosides (Cristina et al., 2022), as in our case (enrofloxacin and marbofloxacin were the drugs of first choice).

In reptiles, captivity can lead to a number of negative health effects, including immunosuppression and hormonal imbalance. Prolonged stress may result in behavioural issues, reproductive problems, and increased vulnerability to diseases that can compromise their overall well-being (Waeyenberge et al., 2018). Proper treatment of skin diseases in reptiles can begin after an accurate diagnosis has been made and, in the case of conditionally pathogenic agents, after the husbandry problems that were a prerequisite for the disease have been corrected (Palmeiro and Roberts, 2013).

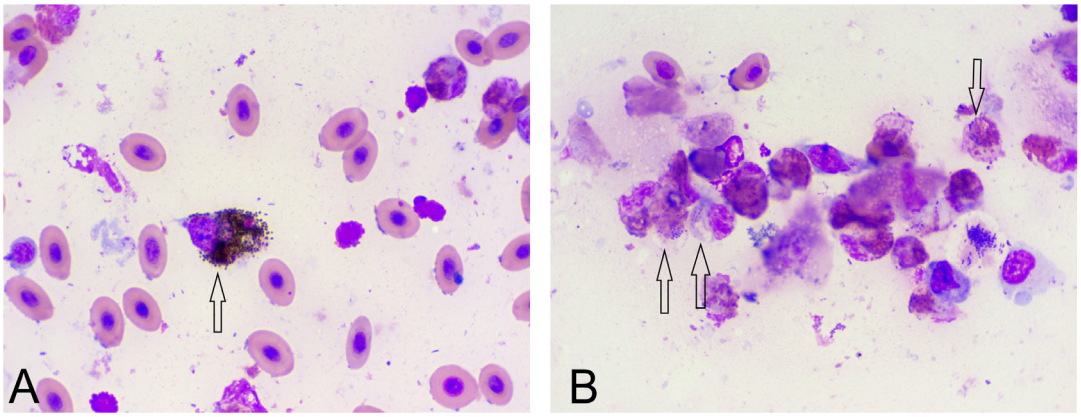

Figure 2. Cytologic impression smears in a Dalmatian wall lizard (*Podarcis melisellensis*). May-Grünwald Giemsa stain. (A) One individualised round cell with intracytoplasmic brown-black pigmented granules and a few identical extracellular granules – melanophore (arrow); (B) Arrows show many intracytoplasmic rod-shaped bacteria within heterophils and one monocyte (100x objective). Photos by Siniša Faraguna.

There are no data on skin infections of the Dalmatian Wall Lizard in the wild or in captivity. Captivity can have significant effects on the immune status of wild lizards, potentially leading to immunosuppression due to stress, altered diets, and limited environmental enrichment (Fischer and Romero, 2019). This weakened immune response may increase susceptibility to infections, such as those caused by *Pseudomonas* spp., which could proliferate more rapidly in compromised individuals and ultimately impact their health and growth (Moser et al., 2021). Current data on the sensitivity of pathogens and on the appropriate use of antibiotics and/or chemotherapeutic agents for the treatment of infectious dermatitis in lizards are limited and available only for a few species. In general, no data are available for the treatment of most lizard species from the wild, so the use of certain preparations is not safe or confirmed due to fully unknown dosage, pharmacokinetics and pharmacodynamics.

Based on the results of the microbiological examination and the antibiogram, treatment was started on the third day after sampling with marbofloxacin (Carpenter and Harms, 2022) at a dose of 2.0 mg/kg body weight (Marfloxin, 100 mg/mL, Krka, Novo Mesto, Slovenia), intramuscular, diluted with sterile physiological saline (0.9 % NaCl) 1:5, every 24 hours for 7 days following complete withdraw of infection.

In conclusion, we can assume that in this case the increased humidity and poor ventilation in the terrarium may have been a prerequisite for a local bilateral skin infection of the buccal region with the bacterium *P. aeruginosa* in the *P. melisellensis* individual, and that the parenteral treatment described above was successful.

Acknowledgments. The *Podarcis melisellensis* male in this report is an additional lizard that was left over after an experiment as part of the BOLDeR project. All necessary permits were obtained for this project: a permit for collecting lizards in the wild from the Ministry of Environmental Protection and Green Transition (No.: 517-10-1-1-21-4) and a permit from the Ethics Committee of the Faculty of Science, University of Zagreb (No.: 251-58-10617-23-1094).

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Accepted by Anamarija Žagar