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Skin bacterial microflora of two closely related mountain newts (Salamandridae) – the Yellow-spotted mountain newt *Neurergus derjugini* and the Kaiser's mountain newt *Neurergus kaiseri* – in the wild and in a breeding facility highlight new conservation perspectives

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Microbiome research is important for the identification of factors that are associated with the *ex situ* care of amphibians, such as potential contagious and lethal pathogens. Nevertheless, it can be also used to establish whether newts can adapt to exposure to new microbial communities, which would be important for the success of future reintroductions into the natural habitat. There is no available information regarding the skin flora naturally occurring in mountain newts of the genus *Neurergus*, including the Yellow-spotted mountain newt *Neurergus derjugini* and the Kaiser's mountain newt *Neurergus kaiseri*. In this study, skin bacterial microbiota of wild adults and individuals of both species from a captive-breeding facility (CBF) were compared. Four bacteria that naturally occur on the skin of wild adult *N. derjugini* were identified (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus, Escherichia coli*). Six bacteria were positively detected on the skin of wild adult *N. kaiseri* [*S. aureus*, *B. subtilis*, *B. cereus*, *E. coli*, *Rhodococcus equi*, *Klebsiella* (*Enterobacter*) *aerogenes*]. Our results indicate that the skin microbiota of F1 offspring (≤ 2 months of age) from the CBF did not correspond exactly to the microbial community identified in wild adult *N. derjugini*. However, ≥ 75% of the bacteria found in older individuals (≥ 1 year of age) living at the CBF over the long term corresponded to those of their wild counterparts. It would appear that post-metamorphic and adults of both species might be better able to resist and adapt to natural microbiota than larvae.

Key-words: amphibians; captive management; microbiome; mountain newt; reintroduction; skin flora.

INTRODUCTION

Since the 1980s, the global decline of wildlife populations has occurred at an astounding rate, with a significant rate of extinction in amphibian communities (Stuart *et al.*, 2004; MacCulloch, 2008; Hernandez, 2016). This well-known phenomenon has been unanimously acknowledged and can be related to multiple anthropogenic and natural factors, such as overexploitation, habitat loss (Cushman, 2006), climate change (Blaustein *et al.*, 2010), the introduction of non-native species, pollution (Blaustein & Kiesecker, 2002) and infectious disease (Hayes *et al.*, 2010). For many threatened species, when survival in the natural habitat is no longer possible,

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ex situ facilities are the only available way to prevent total extinction (Gagliardo *et al.*, 2008; Raffaëlli, 2013). Thus, the conservation of nature and species has turned into a scientific discipline that primarily makes use of two techniques: *ex situ* (off-site) and *in situ* (on-site) initiatives. Although breeding and keeping animal species in human care can be implemented in our societies, reintroduction initiatives are not always effective and tend to be difficult to achieve (Hernandez, 2016). Breeding programmes are an *ex situ* conservation practice advocated by the International Union for Conservation of Nature (IUCN) and may be the most appropriate remedy when it comes to preventing the total extinction of a threatened species. Breeding a threatened species in captivity may be fundamental if it is going to be possible to restore species into natural habitats through reintroductions.

Managing species in *ex situ* facilities can not only permanently deteriorate the host's genome but also alter the symbiotic microbial communities associated with these organisms. It is commonly known that *ex situ* facilities can affect the microbiome of animals and consequently could reduce the chances for conservation achievements, including successful reintroductions into native and natural habitat (Redford *et al.*, 2012; Kohl *et al.*, 2014). The study of microbial communities can be used to identify factors that are associated with captivity and how these impact effective conservation programmes. Microbiome research is especially important for amphibian species, which are facing a global population declines and extinctions from chytridiomycosis (caused by the fungal pathogen *Batrachochytrium* spp). *Batrachochytrium* spp damage the keratin layer in the surface of the skin, affecting the ability of amphibians to respire and intake water through their permeable skin (Van Rooij *et al.*, 2015). Further studies are needed to identify variations in skin and gut microbiota on *ex situ* individuals and how these differ from conspecifics living in the wild, as well as what impact these variations have on long-term conservation of species (Woodworth *et al.*, 2002; Becker *et al.*, 2014; Jiménez & Sommer, 2017).

Recent studies confirmed that the microbiome of living animals can impact host health directly by influencing metabolism, development, inflammation or behaviour. However, the microbiome may also have an indirect influence on host health during interactions with infectious pathogens (Turnbaugh *et al.*, 2006; Heijtz *et al.*, 2011; Honda & Littman, 2012; Theis *et al.*, 2013; Jani & Briggs, 2014). All amphibians show an important and diverse microbiota community on their permeable skin, which is thin and sensitive to their environment (Duellman & Trueb, 1986; Bataille *et al.*, 2016). Amphibian species are mainly habitat specialists inhabiting specific ecological niches associated with water and humidity (Hernandez, 2016). Thus, a wide range of factors may influence their microbiome–host interactions, modifying genetics, life-history characteristics and the behaviour of the host to wider effects, including the habitat (Ding & Schloss, 2014). Many influencing factors have been discovered in this field of research. Krynak *et al.* (2015, 2016) found that skin microbiomes can be associated with and influenced by water conductivity, ratio of natural to managed land and latitude. Moreover, seasonality has also been correlated with the frog-skin microbiome (Longo *et al.*, 2015). The latter study confirmed significant seasonal changes in skin microbial communities of adult Lowland leopard frogs *Lithobates (Rana) yavapaiensis* in the wild, which had higher richness and abundance in winter when the amphibians were more susceptible to infection by *Batrachochytrium* spp (Longo *et al.*, 2015). Studies of the microbiome–host relationship have shown that antimicrobial skin peptides present, secretions from mucosal membranes and frequency of skin shedding can all fluctuate in the skin of different frog species and individuals (Van Rooij *et al.*, 2015).

Wild populations of amphibians have a relatively high exposure to bacteria through interactions with or transmission through their natural environment, and also with conspecifics and other species (Soler *et al.*, 2010; Walke *et al.*, 2011). In contrast, amphibians living in human care interact with fewer individuals in a very different, less diverse and heterogeneous environment (Becker *et al.*, 2014; Jiménez & Sommer, 2017). Consequently, these amphibians have a lower exposure to a rich variety of bacteria, and individuals tolerate a simpler cutaneous bacterial-community structure, which may make them less resistant to disease and less suitable for reintroductions into their natural habitat (Antwis *et al.*, 2014; Becker *et al.*, 2014). Several studies have tested the positive and non-positive effects of captivity on the microbiome community of amphibians, including the Oriental fire-bellied toad *Bombina orientalis* (Bataille *et al.*, 2016, 2017), Eastern red-backed salamander *Plethodon cinereus* (Loudon *et al.*, 2014), Japanese fire-bellied newt *Cynops pyrrhogaster* (Sabino-Pinto *et al.*, 2016) and Western toad *Anaxyrus boreas* (Kueneman *et al.*, 2016). In all these studies, the amphibians developed a reduced diversity of skin bacteria in contrast with specimens studied in the wild. However, the Panamanian golden frog *Atelopus zeteki* has been also kept in captivity for conservation purposes and revealed a higher bacterial alpha diversity than wild individuals (Becker *et al.*, 2014). Extrapolating from these studies, adding microbiome research to the raft of other conservation studies carried out in amphibians would optimize the success of *ex situ* programmes, safeguarding the health of individuals in human care, especially for breeding and potential future reintroductions.

In 2010, the Mohamed bin Zayed Species Conservation Fund helped the authors to develop and manage the conservation plan for the genus *Neurergus*, including the Critically Endangered Yellow-spotted mountain newt *Neurergus derjugini* (Plate 1)

Plate 1. Yellow-spotted mountain newt *Neurergus derjugini* **in the wild at Kavat, Kermanshah, Iran. Mozafar Sharifi, Razi University.**

Plate 2. Kaiser's mountain newt *Neurergus kaiseri* **in the wild at LabSefid, Khuzestan, Iran. Mozafar Sharifi, Razi University.**

and the Vulnerable Kaiser's mountain newt *Neurergus kaiseri* (Plate 2). This conservation project included the development of a captive-breeding facility (CBF) at Razi University, Kermanshah, Iran. The ultimate goal of the captive-breeding programme is to provide stock and increase the population size of the species across different breeding streams to ensure long-term survival (Vaissi & Sharifi, 2018). Annual breeding of the newts was successful at the CBF, including specimens from a small population that was previously assumed to be locally extinct, and an experimental introduction was carried out. Juveniles bred and reared at the facility were released in a small enclosure (2 m × 2 m × 1 m high) erected over the chosen spring, which had a water discharge

of *c.* 10 litres per second, that contained no free-ranging newts (Sharifi & Vaissi, 2014; Vaissi & Sharifi, 2018). The high rates of mortality associated with abiotic factors in the early years of working with *N. derjugini* and *N. kaiseri* at the CBF, have made it possible to identify and analyse the microbiome structure of the skin of these species over a long period. In this study, for the first time, we assess the microorganisms that naturally occur on the skin of *N. derjugini* living in the wild and compare them with microorganisms found on the skin of F1 offspring reared at the CBF. Additionally, the natural bacterial microflora of the *N. kaiseri* was identified in adults from the wild and compared with those maintained at the breeding facility.

MATERIALS AND METHODS

Species

Neurergus derjugini (previously known as *Neurergus microspilotus*) has been reported in 42 localities in highland streams of the Zagros Mountains in western Iran and eastern Iraq (Afroosheh *et al.*, 2016). This species is listed as Critically Endangered on the IUCN Red List (IUCN, 2018) because of its very small area of occupancy in its breeding streams (< 10 km^2), fragmented habitats, a continuing decline in the extent and quality of its stream habitat, reduced number of subpopulations and individuals associated with habitat degradation, drought, and over-collection of animals for both the national and international pet trade (Sharifi *et al.*, 2009; Raffaëlli, 2013). The breeding streams of *N. derjugini* in the western Zagros mountains have recently been impacted by water pollution, water extraction and severe droughts, which have led to the extirpation of several local populations. Extraction of stream water for use in nearby orchards is also a major threat to this species (Sharifi *et al.*, 2009).

The Kaiser's mountain newt is a species endemic to the southern Zagros Range in Iran. To date, *N. kaiseri* had been reported at more than 40 localities (IUCN, 2018). This species has been designated Vulnerable by the IUCN because of its highly fragmented breeding habitat and also because it occupies a small range during its reproductive season (IUCN, 2018). According to the IUCN evaluation, the most serious threats to this species are likely to be illegal trade and the presence of non-native fish as a result of the damming of Dez River, which extends the reservoir close to the known localities of *N. kaiseri*. This species has also been included in Appendix I of the Convention on International Trade of Endangered Species (CITES) (Sharifi *et al.*, 2009). Although the addition of *N. kaiseri* to Appendix I of CITES has banned international trade of this species, collection for national illegal trade continues (Sharifi *et al.*, 2013).

Sampling for bacterial isolation

For bacterial identification of wild newts, 50 adult *N. derjugini* [19.31 (♂♂.♀♀)] were collected from Kavat stream (34°52′N, 046°30′E) during the breeding season (spring 2017) in Kermanshah province, Iran. The sex of each mature individual was determined according to Sharifi *et al.* (2012) and Rastegar-Pouyani *et al.* (2013): males have a fleshy protuberance at the base of the tail, whereas females have a prominent cloaca but without the protuberance (Sharifi *et al.*, 2012; Rastegar-Pouyani *et al.*, 2013). For bacterial identification of newts in human care, known-age individuals that had been maintained and bred at the CBF were studied (Vaissi & Sharifi, 2018). In spring 2017, 20 2 month-old larvae, 15 1 year-old newts, 20 3 year-old newts, 20 5 year-old newts and 40 7 year-old *N. derjugini* were analysed during the breeding season. Thus, different cohorts of individuals were tested for each age class. In addition, 50 adults of *N. kaiseri* [23.27 (♂♂.♀♀)] were collected from Labsefid spring (32°33′N, 048°49′E) in Khuzestan province, Iran, and 25 adults (12.13; > 7 years of age) from the CBF at Razi University were also studied for bacterial

identification during spring 2017. All newts were collected using dip nets.

Each individual collected was assessed for health condition. Following the methods reported in the literature, individuals were placed in distilled water three times and rinsed for 30 seconds each time to remove any bacteria that originated from either the CBF or the natural spring water (Culp *et al.*, 2007). After the rinsing processes were complete, a sterile swab (Copan Diagnostics, Inc.) was used to swab the dorsal and lateral areas of each animal's body (Culp *et al.*, 2007). The range of the dorsal swab extended from the posterior of the head down to the pelvic region. Swabs were then placed in sterile vials for analysis. After swabbing, animals were returned to their site of capture if they had been wild caught or to their enclosures at the CBF.

Bacterial identification

The swabs were placed in Brain Heart Infusion broth and within 24 hours were placed in an incubator at 37°C. Bacteria were cultured separately on four agar plates: blood agar, eosin methylene blue agar, nutrient agar and MacConkey agar, and then incubated for 24 hours at 37°C. These conditions made it possible to differentiate bacteria and confirm other tests. In the next step, based on macroscopic and microscopic appearance, the developed colonies were selected from each sample. Standard bacteriological and biochemical procedures were used to identify the pure isolates on the basis of staining reaction, colony morphology, and the cultural and biochemical character of the isolates (Quinn *et al.*, 2011). Bacteria were identified or characterized based on the morphology of colonies (size, margin, elevation and colour), Gram stain (Merchand & Packer, 1967), catalase, oxidase, Methyl Red Voges-Proskauer broth (MR-VP), Indole and Triple Sugar Iron (TSI) tests for sugar fermentation (Cheesbrough, 2006). Biochemical tests used to identify Gram-negative bacteria were the H_2S-Indole test, MR-VP, Simmons' citrate agar, TSI, urea broth base and Salmonella Shigella Agar. For Gram-positive bacteria, the biochemical tests used were litmus milk, nitrate broth, gelatin agar medium, urea broth base, carbohydrate fermentation tests, Mannitol Salt Agar. Finally, the Gram-positive and Gram-negative bacteria were incubated for 24 hours at 37°C.

RESULTS

Table 1 shows the individual bacterial species isolated from the 19.31 wild adult *N. derjugini*, as well as the Mannitol Salt Agar results for 115 larvae and newts in different age groups originating from the CBF (i.e. 2 month-old larvae, and newts of 1, 3, 5 and 7 years). Positive identifications were made for four bacteria that naturally occur on the skin in the wild: that is, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and *Escherichia coli*. Isolated microbes for these four bacteria were also identified in the 7 year-old newts from the CBF, while a range of these microbes were isolated from different age cohorts, along with *Pseudomonas aeruginosa* and *Salmonella arizonae* (Table 1).

For *N. kaiseri*, five bacterial species were isolated from both the 23.27 wild adults and 12.13 adults (> 7 years of age) originating from the CBF (i.e. *S. aureus*, *B. subtilis*, *B. cereus*, *E. coli* and *Rhodococcus equi*) (Table 1). Another bacteria, *Klebsiella (Enterobacter) aerogenes*, was positively identified in the wild individuals but not found on those from the CBF, while *S. arizonae* was isolated only from CBF adults (Table 1).

DISCUSSION

Many reports have revealed the major loss of amphibian populations across the globe (Alford *et al.*, 2001; Blaustein *et al.*, 2001; Stuart *et al.*, 2004; Raffaëlli, 2013; O'Hanlon *et al.*, 2018). More than 41% of amphibian species are considered threatened, including 54·7% of the Urodela group commonly known as newts and

SPECIES	NATURAL HABITAT	CAPTIVE-BREEDING FACILITY (CBF): AGES				
		2 MONTHS	1 YEAR	3 YEARS	5 YEARS	ADULTS 7 YEARS
Yellow-spotted mountain newt						
Neurergus derjugini	*Staphylococcus aureus*		*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*
	Bacillus subtilis				*B. subtilis*	*B. subtilis*
	Bacillus cereus	*B. cereus*	*B. cereus*	*B. cereus*		*B. cereus*
	Escherichia coli		*E. coli*	*E. coli*	*E. coli*	*E. coli*
		Pseudomonas aeruginosa		*Salmonella arizonae*		
Kaiser's mountain newt						
Neurergus kaiseri	*S. aureus*					*S. aureus*
	B. subtilis					*B. subtilis*
	B. cereus					*B. cereus*
	E. coli					*E. coli*
	Rhodococcus equi					*R. equi*
	Klebsiella aerogenes					
						S. arizonae

Table 1. Positively identified skin microflora isolated from the skin of Yellow-spotted mountain newt *Neurergus derjugini* **and Kaiser's mountain newt** *Neurergus kaiseri* **in the wild and at the captive-breeding facility (CBF) at Razi University, Kermanshah, Iran. Different cohorts were monitored at different ages.** *Neurergus derjugini:* **natural habitat (wild),** n **= 19.31 (♂.♀) adults; CBF,** n **= 20 2 month-old larvae,** n **= 15 1 year-old newts,** n **= 20 3 year-old newts,** n **= 20 5 year-old newts,** n **= 40 7 year-old newts.** *Neurergus kaiseri***: natural habitat (wild),** n **= 23.27 adults; CBF,** n **= 12.13 adults (> 7 years of age).**

salamanders (Hernandez, 2016). Despite a widely recognized need for microbiome research to be placed in a more ecological context – especially as it applies to wildlife conservation – few efforts have been made to integrate these fields, especially in a way that might actually address current management practices (Trevelline *et al.*, 2019). The microbiota of amphibian skin is used as a defence mechanism against infections (Becker *et al.*, 2014; Passos *et al.*, 2018). Therefore, the proper functioning of this symbiotic interaction between bacteria and amphibians is vital for amphibians in human care, especially those that are destined to be released into the wild (Passos *et al.*, 2018). Lack of a robust, native microbial community may underlie not only poor animal health in captivity but also the low success rate of some reintroduction programmes (Trevelline *et al.*, 2019). Comparing the skin microbiota of wild and captive newts of the same species should provide a better understanding of whether captive-bred newts are fit for reintroduction.

In this study, we investigated and compared the bacterial microbiota on the skin of wild and captive-bred individuals of two threatened species of *Neurergus*. The results indicate that the skin microbiota of *N. derjugini* larvae (offspring F1) and 2 month-old newts at the CBF was different from that of adult newts in their natural habitat. However, it is worth noting that wild larvae of these species were not tested for skin bacterial microbiota. If wild larvae had been tested there may have been different results. For future studies, we recommend that the natural microbiome at larval stages is assessed for both *N. derjugini* and *N. kaiseri*. One species of bacteria (*B. cereus*) was present in almost all age classes of newts originating from the CBF and was also found in wild individuals.

Various age cohorts of newts from the CBF (i.e. 1, 3 and 5 year-old newts) shared ≥ 75% of the bacteria present in the wild individuals. However, *P. aeruginosa* (2 month-old larvae at CBF) and *S. arizonae* (3 year-old adults at CBF) were unique bacteria not found in the natural habitat of *N. derjugini*. These differences in microbiota are likely to be the result of environmental factors, such as high humidity, high temperature and an alkaline pH (Grice & Segre, 2011). Consequently, our findings confirm that microbiome research is important in terms of long-term conservation planning for newts and salamanders. The authors wish to encourage more microbiome research, in order to identify and improve our understanding of the poorly known factors associated with amphibians in human care, and enhance the potential for successful *ex situ* programmes in the future.

The fact that a majority of the microbes were retained in the *ex situ* facilities suggests that either microbes are transmitted by vertical or pseudo-vertical transmission or they are abundant in a broad range of environments. Vertical transmission occurs when microorganisms are transferred from parents to offspring newts (Bright & Bulgheresi, 2010). In our study, this seems to be unlikely in *N. derjugini* because parents had no contact with offspring after laying eggs. At the CBF, eggs were kept away from adults in order to prevent cannibalism (Vaissi & Sharifi, 2016). However, various activities at the CBF, such as monitoring, feeding and cleaning enclosures, may potentially increase the possibility of vertical transmission of skin bacteria from parents to larvae and post-metamorphic newts, and this requires more precautions and strict biosecurity measures. Moreover, a similar study indicated that skin bacteria can be vertically transmitted from amphibian parents to embryos in some species (e.g. Bare-hearted glass frog *Hyalinobatrachium colymbiphyllum*) (Walke *et al.*, 2011). It has also been suggested that bacteria from the skin of parents could be transmitted into the water within the enclosure (Becker *et al.*, 2014). These bacteria could remain in enclosures after the parents are removed and may subsequently colonize the skin of offspring.

It is important to consider the time that an individual is kept in captivity because this can affect the bacterial community of the skin, despite animals living under the same conditions (Bataille *et al.*, 2016; Kueneman *et al.*, 2016). A study of Oriental fire-bellied toads *B. orientalis* confirmed that individuals in human care for 4 months had a less rich and more homogeneous bacterial community than those kept for 16 months (Bataille *et al.*, 2016). It has been suggested that these differences may be attributable to the different origins of the two populations (Walke *et al.*, 2014), the length of time in captivity or other unknown factors, including the environment in which the toads were kept (Loudon *et al.*, 2014). However, in the Bataille *et al.* (2016) study, levels of bacterial richness and diversity, and bacterial-community composition, were more similar among the captive populations compared to the wild population sampled. Thus, these authors believe in microbiome convergence (Bataille *et al.*, 2016). Other studies have also demonstrated that the bacterial communities of captive amphibians become more similar to their natural environment with time spent in captivity (Becker *et al.*, 2014). According to Becker *et al.* (2014), the species richness, phylogenetic diversity and community structure of the skin microbiota were significantly different between wild and captive golden frogs. However, after living *c.* 8 years in human care, the offspring of the original captive golden frogs still shared 70% of their microbial community with wild frogs (Becker *et al.*, 2014). This same pattern has been seen in other animals managed under long-term captive conditions [Black howler monkeys *Alouatta pigra* (Nakamura *et al.*, 2011), Grizzly bears *Ursus arctos* (Schwab *et al.*, 2011), Capercaillie *Tetrao urogallus* (Wienemann *et al.*, 2011)]. In contrast,

studies of marine sponges maintained in facilities for short periods of time (> 6 months) had very similar surface microbial communities to wild-caught sponges (Gerce *et al.*, 2009; Webster *et al.*, 2011). However, after 12 months in human care, sponges had a very different symbiotic community structure when compared with wild-caught sponges, whereby many wild-associated microbes had been lost altogether and new or rare microbes had become dominant (Webster *et al.*, 2011).

Our study shows that during short periods of time (≤ 2 months) only 25% of the microbial community had similar surface microbial communities to wild-caught newts (*B. cereus*) but in the long term (≥ 12 months) the microbial community had very similar surface microbial communities to wild-caught newts (*S. aureus*, *B. subtilis*, *B. cereus* and *E. coli*). Overall, our results demonstrate that breeding in human care does not appear to alter the structure of the microbial community on *N. derjugini* or *N. kaiseri* during long-term residence at the CBF. New lines of research will investigate whether it is likely (or not) that the (re)introduction of *N. derjugini* and *N. kaiseri* to the native habitat will affect their skin-associated microbial community.

Several studies have attempted to identify the natural flora associated with amphibians. Barra *et al.* (1998) identified four common members of the natural skin flora [*Enterobacter* (*Pantoea*) *agglomerans*, *Aeromonas hydrophila*, *Klebsiella pneumoniae* and *Acinetobacter junii*] of frogs (European frog *Rana esculenta*, European common frog *Rana temporaria* and Oriental fire-bellied toad *B. orientalis*). In similar research, Harris *et al.* (2006) demonstrated that eight bacterial genera which are part of the natural flora of the Eastern red-backed salamander *P. cinereus* and the Four-toed salamander *Hemidactylium scutatum* can inhibit growth *in vitro* of *Batrachochytrium dendrobatidis* (*Bd*) the chytrid fungus associated with amphibian declines. Furthermore, Culp *et al.* (2007) isolated the natural bacterial flora on the skin of apparently healthy wild adult Eastern newts *Notophthalmus viridescens*, larval American bullfrogs *Lithobates catesbeianus* and Eastern red-back salamanders *P. cinereus* living in their natural habitats within Virginia, USA. These authors identified five bacterial species associated with eastern newts (*Raoultella terrigena, Agrobacterium radiobacter, Flavimonas oryzihabitans, Chryseomonas luteola* and *Aeromonas hydrophila caviae*), three bacterial species in bullfrogs (*Pseudomonas fluorescens, Staphylococcus epidermidis* and *Microbacterium laevaniformans*), and four bacterial species (*Flavobacterium johnsoniae, B. cereus, P. fluorescens* and *Microbacterium testaceum*) and one yeast (*Candida molishiana*) in Red-backed salamanders (Culp *et al.*, 2007). The data from these studies have started to provide baseline reference values for the natural skin flora that researchers could expect to find on these species.

At the time of writing, there is no information available regarding the natural skin flora of newts within the genus *Neurergus*. This study has identified four species of microflora that routinely occur on the skin of wild *N. derjugini* (*S. aureus*, *B. subtilis*, *B. cereus* and *E. coli*) and six on the skin of wild *N. kaiseri* (*S. aureus*, *B. subtilis*, *B. cereus*, *R. equi*, *E. coli* and *K. aerogenes*). Furthermore, the skin bacterial microbiota of wild and captive *N. derjugini* and *N. kaiseri* were compared (Table 1). The study of skin-associated microbiome has emerged as an important and promising new field of research in amphibian conservation, with potential applications to disease management, particularly as these relate to the lethal fungus pathogens *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bs*) (Lauer *et al.*, 2008; Lam *et al.*, 2010; Bletz *et al.*, 2013). Our findings demonstrate that recognition of the microbiome can be an effective tool for conservation programmes. While our data are still limited and more-detailed studies are required (including Illumina sequencing), these results indicate that

incorporating microbiome assessments routinely into conservation management plans may enhance the achievements of *ex situ* and *in situ* programmes, especially in the case of threatened and sensitive amphibian species.

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PRODUCT MENTIONED IN THE TEXT

Sterile swab: manufactured by North American Distribution, Copan Diagnostics, Inc., Murrieta, CA 92562, USA.

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