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## Accepted Manuscript

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Authors: R. Devloo, A. Martel, T. Hellebuyck, K. Vranckx, F. Haesebrouck, F. Pasmans



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**Bearded dragons (*Pogona vitticeps*) asymptotically infected with *Devriesea agamarum*  
are a source of persistent clinical infection in captive colonies of dab lizards (*Uromastyx*  
sp.)**

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26 **Abstract**

27

28 *Devriesea agamarum* causes dermatitis and septicaemia in a variety of lizards, notably those  
29 belonging to the genus *Uromastyx*, whereas other species such as bearded dragons (*Pogona*  
30 *vitticeps*) seem to be asymptomatic carriers. Using amplified fragment length polymorphism  
31 (AFLP), the relatedness between 69 *D. agamarum* isolates was examined. The isolates  
32 derived from 44 diseased lizards, of which 31 belonged to the genus *Uromastyx*, and from 25  
33 healthy lizards, of which 21 were bearded dragons. Eight AFLP genotypes were obtained,  
34 four of which comprised 93% of the isolates. These four genotypes were each present in 2, 2,  
35 8 and 13 different captive colonies. Up to three genotypes were isolated from a single infected  
36 colony simultaneously. On two occasions, the same genotype was found in healthy bearded  
37 dragons and diseased *Uromastyx* lizards from the same colony, confirming the role of the  
38 former as an asymptomatic source of infection for the latter. Two genotypes, comprising 12  
39 isolates, were exclusively associated with diseased *Uromastyx* lizards, suggesting strain  
40 dependent host adaptation. Finally, *D. agamarum* was shown to be able to persist for at least  
41 seven years in a lizard colony, persistently causing severe disease in several lizard species.

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43

44 **Key words**

45 *Devriesea agamarum*, reptile, AFLP, animal pathogens, bacterial infections, bacterial  
46 typing

47

48

## 49 **Introduction**

50  
51 Dermatitis and septicemia caused by *Devriesea agamarum* poses a significant health  
52 problem in captive lizards (Hellebuyck et al., 2009a). Most often, *Devriesea* associated  
53 disease in a collection of lizards is a chronic problem, which, if left untreated, persists for  
54 several years and compromises captive maintenance, especially of dab lizard species (genus  
55 *Uromastyx*). Whether the occurrence and persistence of this disease in a collection is caused  
56 by a single endemic strain or by multiple strains is not known. Besides, whereas certain  
57 saurian taxa such as dab lizards (*Uromastyx* sp.) seem predisposed to severe clinical infection,  
58 the bacterium is frequently isolated from the oral cavity of healthy bearded dragons (*Pogona*  
59 *vitticeps*) (Hellebuyck et al., 2009a). This may be due to a difference in host sensitivity or to a  
60 difference in bacterial strain virulence.

61 The aim of this study was to determine the relatedness between *D. agamarum* strains from  
62 healthy and diseased lizards in captivity using amplified fragment length polymorphism  
63 (AFLP).

## 65 **Materials and methods**

### 67 *Devriesea agamarum* strains

68 Sixty nine *D. agamarum* isolates were obtained from patients showing dermatitis, cheilitis  
69 and/or septicemia presented at the Faculty of Veterinary Medicine, Ghent University,  
70 Belgium and from convenience sampling of captive collections. The 69 lizards, belonging to  
71 the genera *Uromastyx* (31), *Pogona* (21), *Crotaphytus* (8), *Agama* (4), *Laudakia* (4) and  
72 *Eublepharis* (1), representing 9 saurian species and 23 different collections (Table 1). A  
73 collection is defined as one or more lizards belonging to the same owner and kept at the same

74 locality. Samples derived from lesions from 44 diseased lizards, of which 31 belonged to the  
75 genus *Uromastyx*, and from the oral cavity of 25 healthy lizards of which 21 were bearded  
76 dragons (*Pogona vitticeps*). *D. agamarum* was isolated on sheep blood agar containing colistin  
77 and nalidixic acid after 1-3 days of incubation at 30°C. Identification to species level was  
78 done as described in Martel et al.(Martel et al., 2008).

79

80 *AFLP and PCRDNA* of *D. agamarum* isolates was extracted using Easy DNA kit  
81 (Invitrogen).

82 Restriction endonuclease digestion and adapter ligation for AFLP and PCR amplification  
83 were performed using the methods of Ceelen et al.(Ceelen et al., 2006). For the restriction PstI  
84 was used. The sequences of the adapter oligonucleotides and the primers for the PCR were  
85 described in the article of De Zoysa and Efdtratio (De Zoysa and Efstratiou, 2000).

86

87 *Gel electrophoresis*

88 The amplified products were separated on 1.5% agarose gel by unidirectional electrophoresis  
89 using 0.5X Tris-burate-EDTA buffer, stained with gel red and visualized on a UV  
90 transilluminator. Fragment size was determined by comparison with 100 bp DNA ladder  
91 (Fermentas).

92

93 *Analysis of AFLP profiles*

94 The pictures of the gels were imported as TIFF-files into Bionumerics version 4.6 (Applied  
95 Maths, Sint-Martens-Latem, Belgium) and a similarity matrix was calculated according to the  
96 DICE algorithm using optimization settings and tolerance level of 1.06. A UPGMA  
97 (Unweighted Pair Group Method with Arithmetic Mean) dendrogram was constructed based  
98 on the average similarity matrices of two replicates. The strains were classified in the same

99 AFLP type if the relatedness was higher than 70%. A three dimensional visualisation was  
100 created using the multidimensional scaling tool based on the average similarity matrix.

101

## 102 **Results**

103

104 Results are summarized in Table 1 and Figures 1 and 2. Eight distinct AFLP types (A-H) were  
105 obtained. The types obtained from two independent experiments showed high similarity. The  
106 four AFLP types A, B, C and D, represented by 28, 24, 9 and 3 isolates respectively,  
107 comprised 93% of all isolates and derived from 13, 8, 2 and 2 collections respectively. Up to  
108 three different AFLP types were isolated from diseased animals from a single collection  
109 (collection B). AFLP types A and B were isolated both from diseased and healthy animals in  
110 3 and 1 collections respectively. From one animal there is an isolate of the oral cavity (isolate  
111 48) and cheilitis (isolate 50.1). In this case, the same AFLP type A was found. Strains  
112 belonging to the closely related AFLP types C and D were isolated exclusively from diseased  
113 *Uromastyx* lizards.

114 Exchange of animals could be demonstrated between following collections: A, D, H, I and R  
115 received animals that were previously kept in collection B. The AFLP types found in  
116 collection B (type A, B and C) were also present in these other collections. It is possible that  
117 the animals of these collections were infected by the animals from collection B.

118

119

## 120 **Discussion**

121

122 The 8 AFLP types obtained in this study show pronounced variation between *D. agamarum*  
123 strains isolated from lizards. Up to three strains were isolated from a single captive colony

124 simultaneously. The majority of the isolates belonged to two AFLP types (A and B), that are  
125 widespread over different collections of lizards. Interestingly, both types were isolated from  
126 diseased and healthy animals. Since most of the lizards of the genera *Uromastix*, *Laudakia*,  
127 *Agama* and *Crotaphytus* that were infected with one of both AFLP types showed clinical  
128 signs of dermatitis and/or septicaemia, types A and B seem to clinically affect a wide variety  
129 of lizards. However, these AFLP types were also highly prevalent in the oral cavity of  
130 asymptomatic bearded dragons. Disease associated with this bacterium is very rare in this  
131 species. In contrast, *D. agamarum* is usually absent from the oral cavity of healthy *Uromastix*  
132 but highly prevalent in diseased animals from this genus (Hellebuyck et al., 2009a). Our  
133 findings thus suggest that AFLP types A and B are highly prevalent as part of the normal oral  
134 microbiota of bearded dragons, which thus constitute an important source of clinical infection  
135 for several other lizard species. Accordingly, the difference in occurrence of *D. agamarum*  
136 associated disease in different saurian taxa can be largely attributed to a different host  
137 susceptibility.

138 In contrast with the previous finding, the two closely related AFLP types C and D, comprising  
139 12 isolates, were isolated exclusively from diseased *Uromastix* lizards. Although one of the  
140 collections in which *Uromastix* lizards were infected by type C also harbored bearded  
141 dragons, this AFLP type could not be isolated from the latter. The source of infection for  
142 these types is thus not clear. Possibly, both types represent genotypes, adapted to dab lizards.  
143 Persistence of a single AFLP type during a prolonged period of time is demonstrated in  
144 collection A, from which AFLP type A was isolated over a period of 7 years from diseased  
145 lizards, belonging to three genera and four species. Besides cheilitis and dermatitis, disease in  
146 this colony was characterized by high mortality (Hellebuyck et al., 2011). This finding  
147 suggests that a single clone of *D. agamarum* may become endemic in a captive colony of  
148 lizards, persistently causing severe disease. Persistence of the bacterium may be promoted by



149 the presence of asymptomatic carriers. Moreover, *D. agamarum* is highly resistant in the  
150 environment, especially under humid conditions (Hellebuyck et al., 2011). Eliminating *D.*  
151 *agamarum* from lizard collections thus poses a difficult challenge and should include treating  
152 all clinically infected animals and thorough environmental decontamination (Hellebuyck et  
153 al., 2011). Since bearded dragons represent a major reservoir of infection with *D. agamarum*  
154 for dab lizards, we advise against housing these species together. Elimination of *D.*  
155 *agamarum* from infected animals using long term antimicrobial treatment has indeed been  
156 described (Hellebuyck et al., 2009b). The only preventive measure at present to avoid *D.*  
157 *agamarum* associated disease in captive lizards consists of quarantine and entry control of  
158 newly acquired animals (Pasmans et al., 2008).

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185 Table 1: Strains of *Devriesea agamarum* used in this study, their origin, year of isolation and AFLP  
186 type. Isolates obtained from diseased animals are shaded. A collection is defined as all lizards  
187 belonging to the same owner and kept at the same locality.

188

189

190 Figure 1. Dendrogram of the obtained AFLP fragments of *Devriesea agamarum* isolates. Cluster  
191 analysis was performed with UPGMA using the DICE algorithm and a tolerance and optimization  
192 level of 1.06%. The strains were classified in the same AFLP type if the relatedness was higher than  
193 70%.

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196 Figure 2. Three dimensional plot of the multidimensional scaling analysis of *Devriesea agamarum*  
197 isolates marked according to disease status (a) and genus (b) of the host. Black dots represent diseased  
198 animals and white dots healthy animals in (a). Black dots represent *Uromastyx sp.*, white dots *Pogona*  
199 *sp.* and grey dots the other lizard species in (b).

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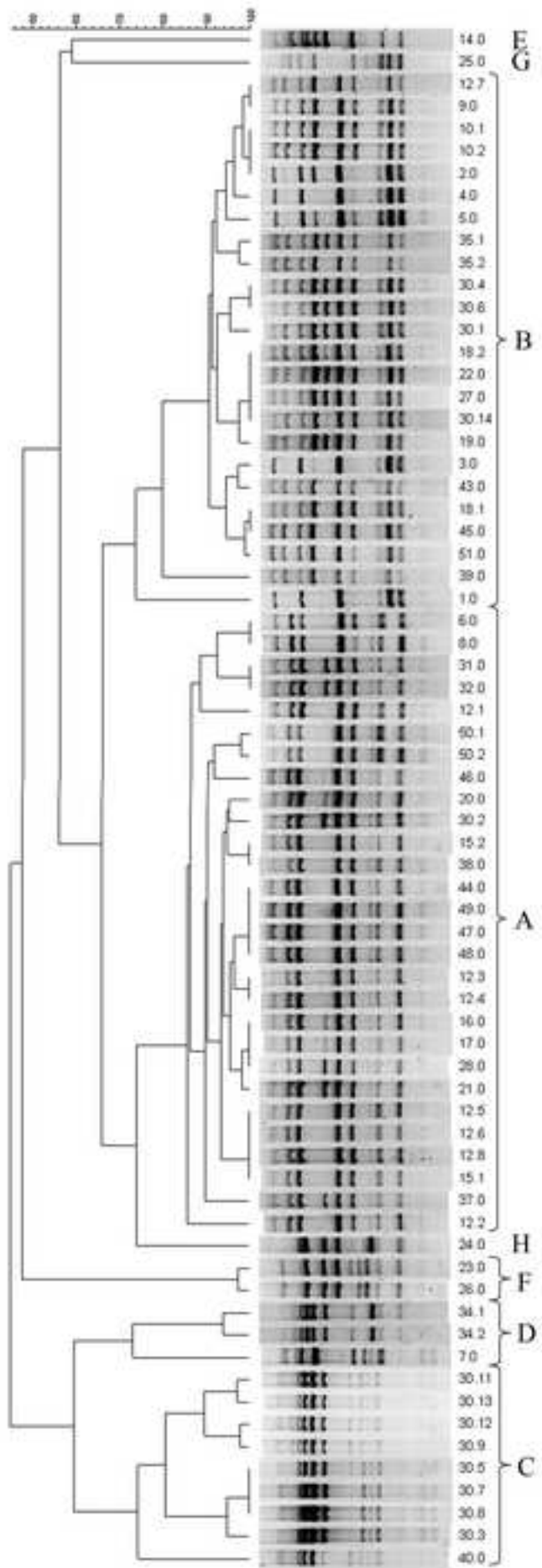
Table 1: Strains of *Devriesea agamarum* used in this study, their origin, year of isolation and AFLP type. Isolates obtained from diseased animals are shaded. A collection is defined as all lizards belonging to the same owner and kept at the same locality.

Collection	Isolate	Species	Origin	Year of isolation	AFLP type
A	1	<i>Uromastyx acanthinura</i>	cheilitis	2005	B
	51	<i>Uromastyx acanthinura</i>	dermatitis/cheilitis	2009	B
	5	<i>Uromastyx geyri</i>	cheilitis	2007	B
	43	<i>Agama impalearis</i>	dermatitis/liver	2003	B
	10.1	<i>Agama impalearis</i>	liver	2003	B
	10.2	<i>Agama impalearis</i>	liver	2003	B
	45	<i>Agama impalearis</i>	dermatitis/liver	2003	B
	3	<i>Crotaphytus collaris</i>	cheilitis/dermatitis	2007	B
	2	<i>Crotaphytus collaris</i>	cheilitis/dermatitis	2007	B
	4	<i>Crotaphytus collaris</i>	cheilitis/dermatitis	2007	B
B	30.2	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	A
	39	<i>Uromastyx acanthinura</i>	cheilitis	2009	B
	30.14	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	B
	30.6	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	B
	30.4	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	B

	30.1	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	B
	30.8	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	C
	30.7	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	C
	30.5	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	C
	30.3	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	C
	30.13	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	C
	30.11	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	C
	30.12	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	C
	30.9	<i>Uromastyx dispar</i>	cheilitis/dermatitis	2009	C
	35.2	<i>Uromastyx ornata</i>	Oral cavity	2009	B
	35.1	<i>Pogona vitticeps</i>	Oral cavity	2009	B
C	12.3	<i>Pogona vitticeps</i>	Oral cavity	2008	A
	12.2	<i>Pogona vitticeps</i>	Oral cavity	2008	A
	12.4	<i>Pogona vitticeps</i>	Oral cavity	2008	A
	12.6	<i>Pogona vitticeps</i>	Oral cavity	2008	A
	12.5	<i>Pogona vitticeps</i>	Oral cavity	2008	A
	12.1	<i>Pogona vitticeps</i>	Oral cavity	2008	A
	12.8	<i>Pogona vitticeps</i>	Oral cavity	2008	A
	23	<i>Pogona vitticeps</i>	Oral cavity	2008	F

D	7	<i>Uromastyx acanthinura</i>	cheilitis/dermatitis	2007	D
	28	<i>Eublepharis macularius</i>	Oral cavity	2007	A
E	46	<i>Uromastyx acanthinura</i>	Bone marrow	2005	A
F	24	<i>Pogona vitticeps</i>	Oral cavity	2007	H
G	47	<i>Laudakia stellio</i>	cheilitis	2009	A
	50.2	<i>Laudakia stellio</i>	cheilitis	2009	A
	50.1	<i>Laudakia stellio</i>	cheilitis	2009	A
	48	<i>Laudakia stellio</i>	Oral cavity	2009	A
	49	<i>Pogona vitticeps</i>	Oral cavity	2009	A
H	16	<i>Uromastyx acanthinura</i>	cheilitis	2008	A
	15.2	<i>Pogona vitticeps</i>	Oral cavity	2008	A
	15.1	<i>Pogona vitticeps</i>	Oral cavity	2008	A
I	38	<i>Uromastyx geyri</i>	cheilitis	2009	A
	8	<i>Uromastyx acanthinura</i>	cheilitis	2007	A
J	6	<i>Pogona vitticeps</i>	Oral cavity	2008	A
K	44	<i>Uromastyx dispar</i>	dermatitis/liver	2006	A
L	21	<i>Crotaphytus collaris</i>	dermatitis	2008	A
	27	<i>Crotaphytus collaris</i>	sepsis/liver	2008	B
	20	<i>Crotaphytus collaris</i>	Oral cavity	2008	A

M	32	<i>Pogona vitticeps</i>	Oral cavity	2008	A
	31	<i>Pogona vitticeps</i>	Oral cavity	2008	A
N	17	<i>Pogona vitticeps</i>	Oral cavity	2007	A
O	37	<i>Pogona vitticeps</i>	Oral cavity	2009	A
P	26	<i>Crotaphytus collaris</i>	dermatitis	2008	F
Q	34.1	<i>Uromastyx acanthinura</i>	abces	2009	D
	34.2	<i>Uromastyx acanthinura</i>	cheilitis	2009	D
R	40	<i>Uromastyx acanthinura</i>	cheilitis/dermatitis	2008	C
	14	<i>Uromastyx acanthinura</i>	abces/dermatitis	2008	E
S	9	<i>Uromastyx ornata</i>	cheilitis	2008	B
	25	<i>Pogona vitticeps</i>	Oral cavity	2008	G
T	18.2	<i>Uromastyx aegyptia</i>	abces	2008	B
	18.1	<i>Uromastyx aegyptia</i>	dermatitis/abces	2008	B
U	22	<i>Crotaphytus collaris</i>	dermatitis	2008	B
V	19	<i>Pogona vitticeps</i>	Oral cavity	2007	B
W	12.7	<i>Pogona vitticeps</i>	Oral cavity	2007	B





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