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Lucek, K. and Lemoine, M. (2012) First record of freshwater fish on the Cape Verdean archipelago. *African Zoology*, 47 (2). pp. 341-344. ISSN 1562-7020

<https://doi.org/10.1080/15627020.2012.11407545>

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1 **Introduced guppies being the first record of freshwater fish on the Cape**
2 **Verdean archipelago**

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4 Kay Lucek^{1,2,4} & Mélissa Lemoine³

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6 ¹ Institute for Ecology & Evolution, Division of Aquatic Ecology &
7 Macroevolution, University of Bern, Baltzerstrasse 6, CH-3012 Bern, Switzerland

8 ² Department of Fish Ecology, EAWAG Center for Ecology, Evolution and
9 Biogeochemistry, Seestrasse 79, CH-6047 Kastanienbaum, Switzerland

10 ³ Institute for Ecology & Evolution, Division of Evolutionary Ecology, University
11 of Bern, Baltzerstrasse 6, CH-3012 Bern, Switzerland

12 ⁴ kay.lucek@eawag.ch

13

14 Key words: Cape Verde, guppy, *Poecilia reticulata*, Macaronesia.

15

16 **Abstract**

17 The Cape Verdean islands form a distinct aquatic freshwater ecoregion
18 characterised mainly by temporal water bodies with an adapted invertebrate
19 community. Freshwater fish were not yet recorded from the archipelago. During
20 a non-exhaustive screen of freshwater bodies on five islands of the archipelago,
21 the first presence of freshwater fish was recorded in an artificial reservoir. Using
22 barcoding sequences, the species was identified to be introduced guppies
23 (*Poecilia reticulata*), a highly invasive species.

24

25

26 **SHORT COMMUNICATION**

27 The Cape Verdean archipelago lies in the middle of the Atlantic Ocean about 500
28 km off the west coast of Africa and consists of ten islands and several islets of
29 volcanic origin (Brown 2004 and Figure 1). Being in the dry belt of the Sahel, the
30 archipelago experiences a tropical climate with low rainfall. Consequently, water
31 exists mainly as temporal streams only flowing after rainfall. Permanent streams
32 only occur on the island of Santo Antão (Hazevoet 1995). However, extended
33 periods of droughts occur regularly during which most water bodies dry out.
34 This unstable environment led to the evolution of a specialised endemic
35 freshwater fauna, consisting of invertebrates only, including dragon flies
36 (Martens 2010) and freshwater snails (Rosa *et al.* 1999). Freshwater fish on the
37 other hand have never been recorded (Brown 2004).

38 The guppy (*Poecilia reticulata* Peters, 1859) is a small benthopelagic fish
39 native to Central and South America (Lindholm *et al.* 2005). Because it is a
40 popular aquarium fish, as well as its use as an agent for mosquito control, this
41 species has been introduced widely and is now found on all continents except
42 Antarctica (Froese & Pauly 2012). The wide environmental tolerance of *P.*
43 *reticulata* such as its resistance to high salinity, allowing colonization through
44 seawater (Chervinski 1994), as well as its high fecundity, makes it a very
45 successful invader (Lindholm *et al.* 2005). In part of its invasive range, the
46 presence of guppies has resulted in a decline of native fish and invertebrate
47 species abundance (Courtenay & Meffe 1989). *P. reticulata* is therefore a
48 possible threat when introduced into new habitats.

49 Between October 7th and 27th in 2011, the authors screened five islands
50 (Boavista, Sal, Santa Antão, São Nicolau and São Vincente) for the presence of

51 freshwater bodies (Figure 1). Screening was performed by hiking transects
52 either along the coast (Sal, Boavista) or along stream channels within valleys. All
53 encountered freshwater bodies were visually inspected for the occurrence of
54 molluscs and fish. Where present, samples were collected using a 20 x 25 cm
55 hand net with a mesh size of 3 mm. Screening included temporal rivers with
56 remaining water ponds, streams and artificial reservoirs. This was performed in
57 a non-exhaustive way and therefore only a small fraction of the potential
58 available habitats were sampled.

59 Freshwater bodies were only found on two islands (Boavista and Santa
60 Antão). Here, only one artificial water reservoir on Santa Antão (17°08.169' N,
61 25°03.973' W) contained freshwater fish (Figure 1). These were later identified
62 as *P. reticulata*, with both adults and juveniles being abundant (length range?).
63 The artificial water reservoir was constructed of concrete (8 m long x 4 m wide x
64 1.5 m deep) with muddy substrate on the bottom and is part of an irrigation
65 system receiving inflowing water from irrigation channels and is further
66 connected to a freshwater stream. No fish were however observed in the streams
67 below the reservoir.

68 Two juveniles were captured with a hand net, sacrificed with an overdose
69 of anaesthetic clove oil and preserved in absolute ethanol for further analysis. To
70 determine species identity of the juvenile fish, standard barcoding primers
71 (LC01490, HCO2198, see Folmer *et al.* 1994), amplifying a part of the
72 mitochondrial cytochrome c oxidase subunit I (COI) gene were used. DNA was
73 extracted using a 10% Chelex solution, following the manufacturers protocol
74 (Biorad, Switzerland). PCR setup was as follows: initial denaturation at 94°C for
75 3 min followed by 35 cycles with 30 sec at 94°C, 30 sec at 48°C and 1 min at 72°C

76 with a final elongation step of 5 min at 72°C. Sequencing was performed on a
77 Beckman Coulter CEQ 8000 capillary system (Beckman Coulter, Switzerland)
78 following the manufacturers instruction.

79 Obtained sequences were aligned using BioEdit 7.1.3 (Hall 1999). A
80 phylogenetic tree was constructed with publicly available barcode sequences of
81 the *Poecilia* genus on BOLD (Ratnasingham & Hebert 2007) in MEGA 4 (Tamura
82 *et al.* 2007). Here, using 654 overlapping base pairs (bp), an unrooted neighbour
83 joining tree was constructed with the implemented pairwise deletion option.
84 Further statistical support was estimated using 1000 bootstrap replicates.

85 Both sequenced individuals showed the same mitochondrial COI
86 haplotype. The obtained sequence was deposited on GenBank (Accession
87 number: JQ734533). In the reconstructed phylogenetic tree, these two
88 individuals formed a significant clade with two other specimens of the species
89 *Poecilia reticulata* (GenBank accession number JN028265 and JN028266, Figure
90 2), which originate from an introduced lineage in Canada. The clade significantly
91 differentiated *P. reticulata* from other species of the *Poecilia* genus.

92 Although we were only able to catch juveniles, the barcoding approach
93 reliably identified the species identity of both individuals as *P. reticulata*.
94 Because malaria is absent from Santa Antão and most other islands (Alves et al.
95 2006), it seems to be unlikely that this species was introduced for mosquito
96 control. Therefore, it is very likely that the population represents an
97 unintentional introduction originating from ornamental fish trade. The ancestral
98 source population could not be identified because only few sequences were
99 available.

100 Introduced species are considered to be a major threat to the local
101 ecosystem of the Cape Verdean islands, with over 300 known invasive species
102 being present (CBA 2007). The introduction of guppies may have negative
103 impacts on the freshwater ecosystem as its biota evolved in the absence of
104 vertebrate predators. However, further studies are needed to assess the actual
105 impact of guppies on the local ecosystem. Given the known invasiveness of
106 guppies, even when genetically depauperated (Lindholm *et. al.* 2005), as well as
107 its ability to compete with other species (Courtenay & Meffe 1989) it seems to be
108 likely that the observed population could colonise a larger range. Here the
109 artificial water reservoirs might play a crucial role as they provide safe refugia
110 even during droughts, which could allow the species to persist. Because the
111 species seemed locally restricted, measures could still be implemented to avoid
112 its further spread.

113

114 **Acknowledgement**

115 We would like to thank M. P. Haesler for assistance with the sequencing. The
116 Cape Verde Honorary Consulate in Basel provided valuable information on the
117 Cape Verde legislation. Two anonymous reviewers provided valuable comments
118 on earlier versions of the manuscript.

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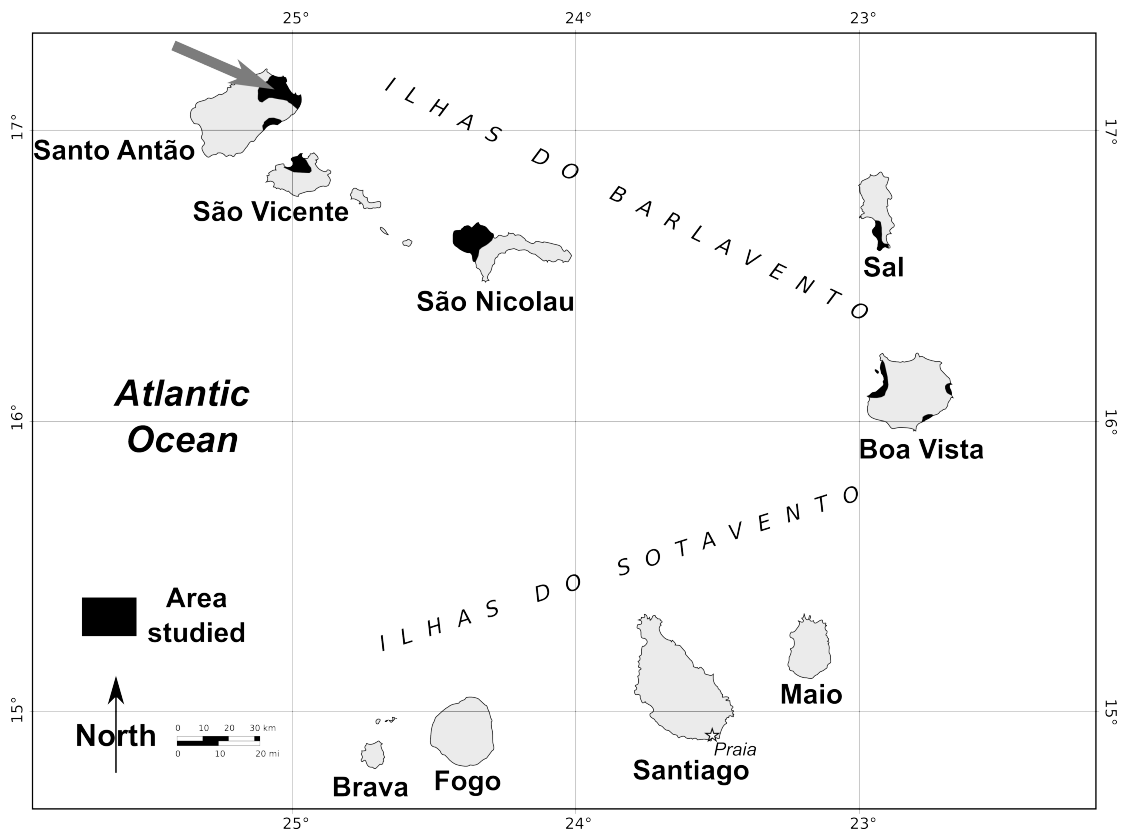
175 **Figure Legends**

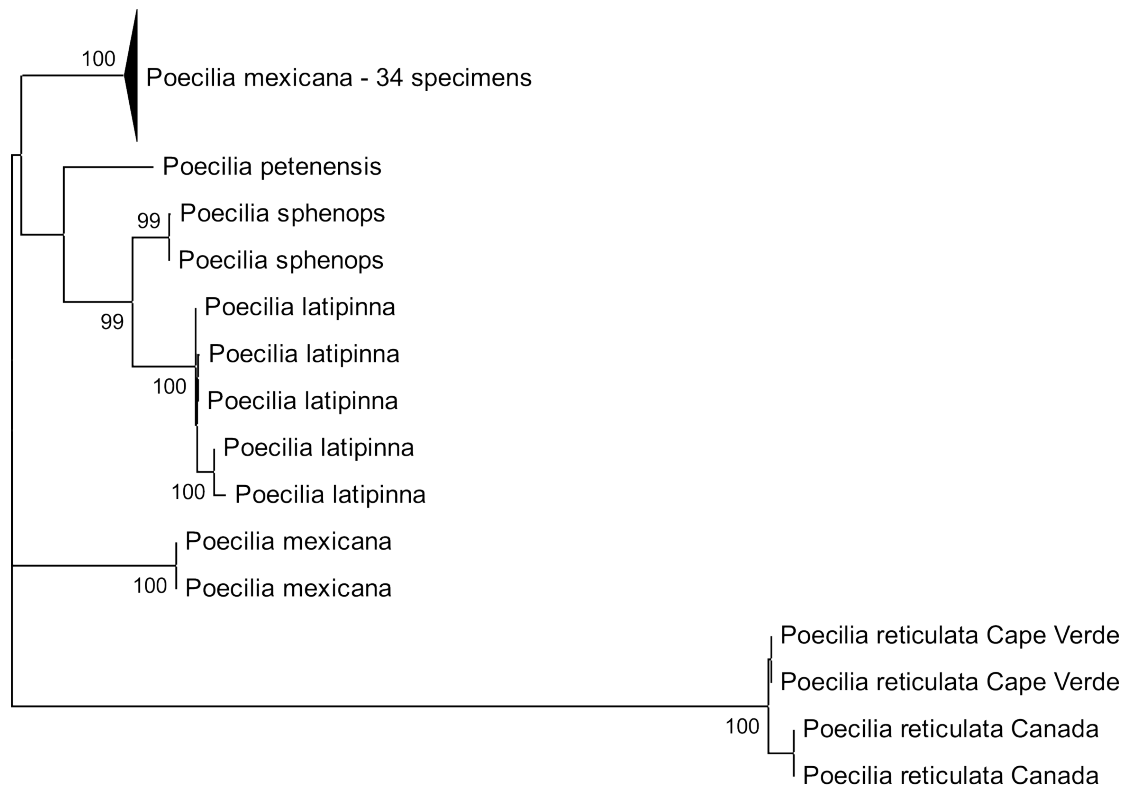
176 Figure 1: Map of the Cape Verdean archipelago (modified from © WIKIMEDIA
177 2011). Black areas indicate the regions investigated in this study. The arrow
178 indicates the only site where *Poecilia reticulata* has been observed.

179

180 Figure 2: Unrooted phylogenetic tree using 654 base pairs of the mitochondrial
181 control region. Statistical support was estimated using 1000 bootstrap
182 replicates. Only values with >90% bootstrap support are indicated next to the
183 branches.

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