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First record of the Manx Shearwater *Puffinus puffinus* in Malta: evidence from morphometric data and DNA analysis.

John J. Borg, Joe Sultana, Petra Heidrich and **Michael Wink**

The breeding range of the Manx Shearwater *Puffinus puffinus* is in the North Atlantic, with the largest colonies found in the British Isles. The majority of the birds migrate to South America; the adults start moving in July followed by the juveniles in September (Cramp and Simmons 1977). Occasionally birds are reported away from their normal route. Up to 9 birds were recovered in Switzerland between 1866 and 1990. All were recovered in September, except one in July (Juillard 1992).

The Manx Shearwater does not venture into the Mediterranean regularly. A Welsh-ringed bird was recovered during its first winter, in February, on the French Mediterranean coast (Blondel & Isenman 1981).

A *Puffinus* shearwater was found at the Freeport at Marsaxlokk Bay, on the SE coast of Malta, on 6 September 1995. On close examination it was identified as a first year Manx Shearwater *Puffinus puffinus*. A blood sample was also taken for DNA analysis.

The plumage was distinctly different from that of the Levantine Shearwater *Puffinus yelkouan.* The head was black with white feathers near the base of the bill, lores and behind the ears forming a crescent shape. The chin and the throat were also white. The upperparts were jet-black and the underparts were white with grey mottling along the flanks and sides of breast. The tail was black, with the undertail coverts white with faint grey markings. Underwings were mostly white with black wingtips and black trailing edges and with grey markings and pattern on carpal and axillaries. The iris was dark brown, the bill black and the feet a deep pink with black markings and blotches. The plumage was fresh with no apparent traces of wear or bleaching - this suggested a recently fledged bird.

The following are the measurements of the bird as compared with an average from 13 birds belonging to the species *Puffinus yelkouan.* Numbers 1-6 denote the different measurements of the bill (see Fig 1):

	wing	tarsus	tail	1	2	3	4	5	6
p.puffinus	231.0	43.2	78.1	32.7	24.3	9.0	6.5	10.0	3.6
p.yelkouan	232.7	46.6	74.0	35.4	27.8	10.6	7.1	11.9	3.2

Table 1. Biometrics of *P.puffinus* and *P.yelkouan* from Malta.

DNA was isolated according to standard protocols as described in Heidrich *et al.* (1995, 1997). The mitochondrial cytochrome *b* gene, which is a good marker gene in Procellariiformes and other birds (Austin 1996, Wink *et al.* 1993a, 1993b, 1996, Heidrich *et al.* 1996, 1997, Wink 1995) was amplified by PCR and sequenced directly (1080 nucleotides). Sequences were aligned with the cytochrome b sequence of *Gallus g. domesticus* (Desjardins and Morais 1990) and analysed with the distance matrix method Neighbour Joining (NJ; MEGA 1.0) based on genetic distances (Kumar et al. 1993). For comparison, we analysed the nucleotide sequences of *Puffinus puffinus*, *P. yelkouan*, *P. mauretanicus*, *P. assimilis* and of other Northern Palaearctic Procellariiformes such as *Calonectris diomedea*, *Bulweria bulwerii*, *Hydrobates pelagicus* and *Oceanodroma castro*.

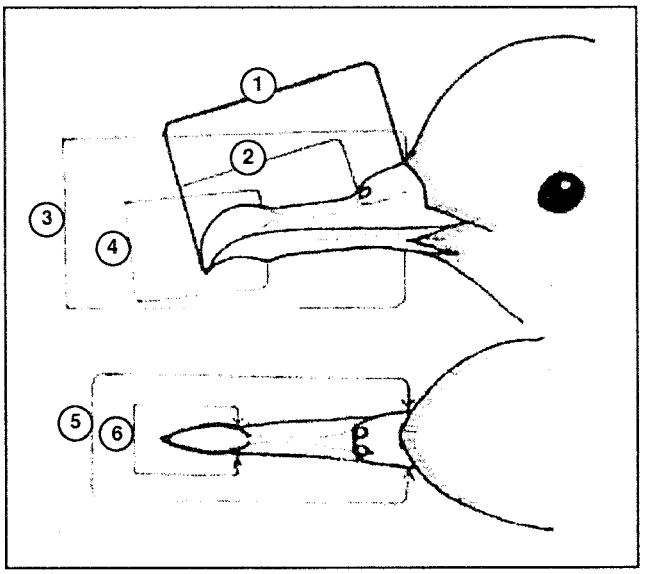

Fig 1. Key to bill measurements

As Fig 2 shows, the bird in question from Malta unequivocally clusters with *P. puffinus* from the North Atlantic (supported by a bootstrap value of 100%). It is evident that this bird does not belong to one of the Mediterranean *Puffinus* species (*yelkouan* or *mauretanicus*). The nucleotide sequence of the Malta bird differs by 2 to 3 nucleotide substitutions from P. puffinus collected in Ireland (Table 2).

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                          111 111 111 122 222 223 333 344 444 444 444 455 556 666 666 667 777 888 889 999
                  12 356 668 000 122 346 912 334 690 367 904 555 666 678 904 590 035 678 990 009 167 990 234
                 697 010 691 258 409 212 262 173 476 305 321 039 256 710 219 210 702 988 890 239 043 147 165

P. assimilis      AAA AAT TCA CCT CCT CTC ATT CAA ATC TTC AAC CCC TCA TCT TCC CCT TAT CAC CTC CCT TTC CTT TAT
P. mauretanicus 1 C.G G.C ... TTC T.C T.. ..C TGG .CC C.T ... TTT .TG CTC C.T .T. ..C TG. ... ..C CCT .CC CGC
P. p. puffinus 1  C.. ... C.. ... ... TCT GC. TG. ..C CC. .G. T.T ... ..C C.T TTC C.C T.. TCT ATC CCT .C. ..C
P. p. puffinus 2  C.. ... C.. ... ... TCT GC. TG. ..C CC. GG. T.T ... ..C C.T TTC C.C T.T TCT ATC CCT .C. ..C
P. ?? Malta       C.. ... C.. ... ... TCT GC. TG. ..C CC. .G. T.T ... ..C CAT TTC C.C T.T TCT ATC CCT .C. .GC
P. yelkouan 1     CC. GGC .TT T.. TTC ... ..C TG. GCC C.. .GT T.T C.G ..C C.T .TC .GC TG. ... ..C CCT .CC CGC
P. yelkouan 3     C.. GGC .T. T.. TTC ... ..C TG. GCC C.. .GT T.T C.G ..C C.T .TC .GC TG. ... ..C CCT TCC CGC
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Table 2. Variable sites in the cytochrome b gene of *Puffinus yelkouan* shearwaters. The unknown bird from Malta is named *P.??* Malta
. base identical to that in the first line.

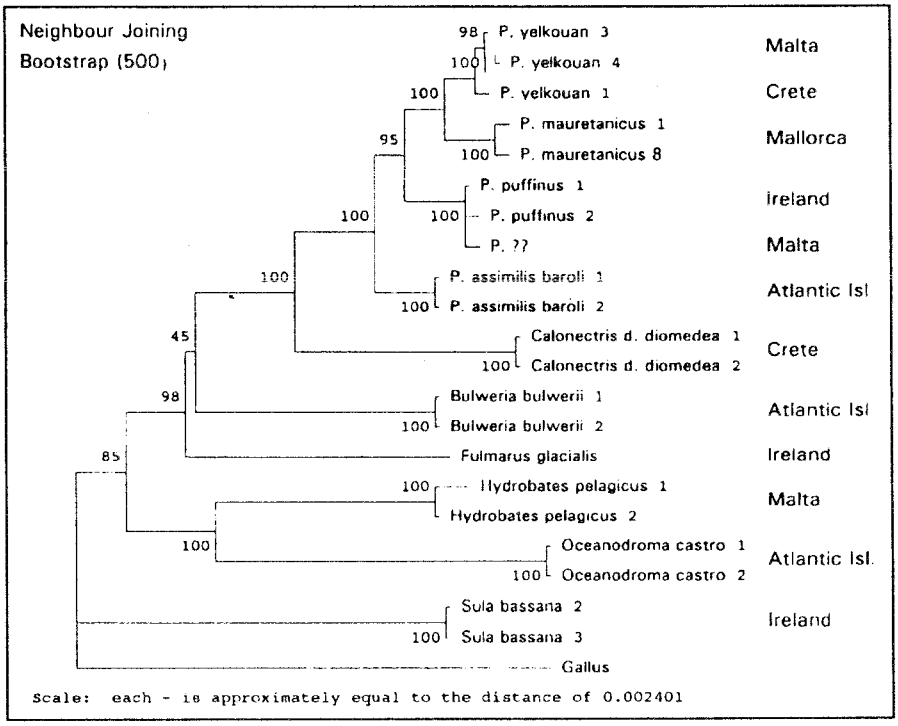

Fig 2. Phylogenetic relationships in *Puffinus* shearwaters and other Procellariiformes based on 1080 bp of the cytochrome b gene. Illustration as a phylogram in which branch lengths are proportional to genetic distances. Bootstrap values are given at each forcation. *Gallus* was used as an outgroup and *Sula bassana* as an unrelated ingroup. Birds from Malta were collected by JB, those from Crete by D Ristow and MW, those from the Atlantic Islands by F Zino, those from Mallorca by J Aguilar, and those from Ireland by MW.
Note that the Manx from Malta (*P.??* Malta) unequivocally clusters with *P.p.puffinus*.

	1	2	3	4	5	6	7
1 *Puffinus assimilis*	-	4.27	3.44	3.62	3.71	4.09	4.18
2 *P. mauretanicus*			3.62	3.81	3.71	2.23	2.23
3 *P. puffinus* 1				0.19	0.28	3.25	3.34
4 *P. puffinus* 2					0.28	3.44	3.53
5 P. ?? Malta						3.34	3.44
6 *P. yelkouan* 1							0.46
7 *P. yelkouan* 2							-

Table 3. Genetic distance (number of nucleotide substitutions) in % between *Puffinus* shearwaters. **P.??** = unknown bird from Malta.

Such small distances (0.2 to 0.3%) are common between shearwater populations and were also encountered between individuals of *P. yelkouan* from Malta and Crete (Table 3).

Because of the substantial genetic (Tables 2 & 3) and morphological differences between the Atlantic *puffinus* and the Mediterranean *mauretanicus* and *yelkouan*, which have been treated as subspecies of *P. puffinus*, we have attributed species rank to them in a recent publication (Heidrich *et al.* 1997).

Figure 2 shows that Shearwaters (*Puffinus, Calonectris*) cluster in a monophyletic clade (bootstrap value 100%) sharing a common ancestor. Sister groups include the Fulmar and Bulwer's Petrel. These petrels and shearwaters, which are grouped in the family Procellariidae are separated by a significant bootstrap value (98%) from Storm Petrels (*H. pelagicus, 0. castro*) of the family Hydrobatidae.

Both the morphological data as well as the DNA analysis results clearly show that the Manx Shearwater in question belongs to the Atlantic *P. puffinus*. This example shows, in addition, that DNA methods and especially DNA sequencing provide a powerful tool to corroborate faunistic observations and biometric data.

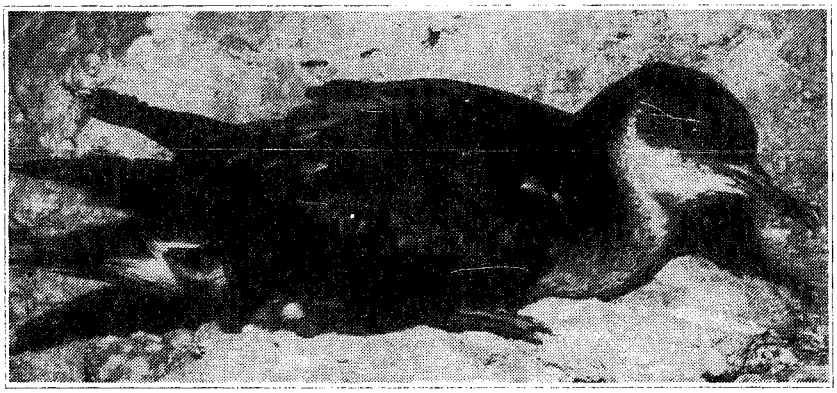

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Fig 3. The bird in question, shortly before release.

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