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On the systematic status of the Cyrenaic Partridge (*Alectoris barbata* Reichenow, 1896)

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Abstract – Some considerations on the morphological features that differentiate *Alectoris barbara barbata* by *A. b. barbara* are exposed and are also reported the results of a genetic investigation performed on historical specimens. Results showed a considerable genetic distance (0.60), certainly enough to consider it an ESU (Evolutionary Significant Unit), but most likely a separate species.

Key-words: *Alectoris barbara barbata*, Cyrenaica, genetic.

INTRODUCTION

The barbary partridge *Alectoris barbara* is widespread in North Africa from Morocco to Egypt and is also present in Sardinia (Cramp & Simmons 1980, Madge & Gowan 2001). Over much of range it has declined due to hunting pressure and habitat degradation (Madge & Gowan 2001, BirdLife International 2004). Locally numerous in Morocco, Algeria and Tunisia, current status in Lybia is unknown and it is perhaps extinct in Egypt (Madge & Gowan 2001). The eastern subspecies *A. barbara barbata*, who lives in the Cyrenaica, was described as a species by Reichenow (1896) and later, as *Caccabis callolaema*, also by Salvadori & Festa (1916). It was also considered as separate species by Ghigi (1923); however, in the recent literature, it is usually listed under subspecies of *Alectoris barbara* (Cramp & Simmons 1980, Madge & Gowan 2001).

The purpose of this paper is to provide some starting points for a correct systematic position of *Alectoris barbata*.

MATERIAL AND METHODS

In addition to the obvious visual comparison of the morphological characteristics of the two taxa we have also measured some morphometric parameters (bill, wing, third primary, tarsus, tail) on subjects preserved in Museums (7 in Museo Civico di Storia Naturale "G. Doria" of Genoa, 17 in Museo Regionale di Scienze Naturali of Turin).

We also analyzed 241 bp from d-loop mtDNA in four *A. barbata* individuals collected in Cyrenaica (Libya) between 1915 and 1922 and preserved in the Museum of Turin. Total genomic DNA was isolated from feathers with NucleoSpin kit (Macherey-Nagel), according to the manufacturer's instructions with the following modifications: feather tips were placed in a lysis buffer for small DNA quantity (FLB buffer; Macherey-Nagel) and exposed to thermal shock in liquid nitrogen. Sequences of hypervariable domain I of mitochondrial DNA control region were amplified by polymerase chain reaction using two PCR primers specifically designed. Amplification was carried by the following fast protocol: (94°C x 2'), 30 cycles at (94°C x 30"), (55°C x30"), (72°C x 30"), and a final extension at 72°C for 10 minutes. Amplicons were sequenced at the "BMR genomics" lab (Padova, Italy). Sequence alignment was performed with Bioedit version 7.0.9 (Hall 1999). The Neighbour-joining method (NJ; Saitou & Nei 1987), clustering pairwise Tamura-Nei's (TN93; Tamura & Nei 1993) genetic distances between haplotypes was performed with MEGA 5.0 (Tamura et al. 2011); support for the internodes in the NJ tree was assessed by bootstrap percentages (BP; Felsenstein 1985) after 1000 resampling steps. We used two sequence from *A. chukar*, *A. rufa* as

outgroups and two sequence *A. barbara* from Sardinia and Tunisia as reference.

RESULTS

Alectoris barbara barbata presented several morphological characters rather different from the other subspecies of Barbary Partridge and in particular: throat and eyebrows dark bluish grey (Fig. 1); feathers of the throat with elongated flattened rachis, with "beard" effect; brown orange collar with greyish patches, not red-brown with white patches; feathers of the flanks with one single thick black streak, there is rarely only a vague hint of a second streak in some feathers (Fig. 2); different appearance of the neck, the eyebrows do not break the collar; even with a few specimens measured, the values of the considered morphometric measures, in particular the tail, are significantly greater than in *A. barbara* (Tab. 1).

Results of genetic test conducted on 4 specimens of *A. barbara barbata* led to the results shown in Fig. 3 (NJ tree with Tamura Nei distance on mtDNA d-loop, 241nucleotides) and in Tab. 2 (TN93 genetic distance). These results indicate that genetic distance between *A. barbara* from Sardinia and Tunisia and *A. barbara* from Cyrenaica was 0.060 (6%).

Figure 1. Differences between *Alectoris barbara barbata* (left) and *A. b. barbara* (right) in the colour of collar and throat and in the shape of the feathers of the throat.

Figure 2. Differences in the pattern of the feathers on the flanks between *Alectoris barbara barbata* (left) and *A. b. barbara* (right).

Table 1. Mean values (± SD) of the considered morphometric measures in individuals of *Alectoris barbara barbara* or *A. barbara barbata* and p values of the comparisons between the two subspecies (Mann-Whitney test).

	A. b. barbara	***A. barbara barbata***	**P** (Mann-Whitney)
Bill	17.77 ± 2.48 (N = 10)	19.04 ± 1.06 (N = 12)	0.012
III Primary	104.94 ± 4.89 (N = 8)	111.17 ± 2.79 (N = 12)	0.006
Tarsus	39.40 ± 3.15 (N = 10)	43.73 ± 2.69 (N = 11)	0.003
Tail	88.70 ± 5.10 (N = 10)	106.33 ± 5.90 (N = 12)	0.00001

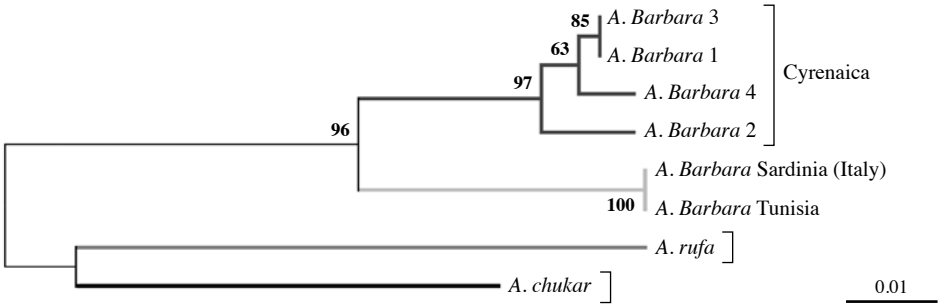

Figure 3. mtDNA d-loop (241 bp) Neighbor Joining tree with Tamura Nei distance (TN93). Four samples from Cyrenaica, Libya (4 individuals sampled between 1915 to 1922, Regional Museum of Natural Sciences, Turin) were analyzed. Two sequence of *A. barbara* from Tunisia and Sardinia were used as the reference and two sequences of *A. rufa* and *A. chukar* were used as outgroups. Support for the internodes was assessed by bootstrap percentages after 1000 resampling steps.

Table 2. Tamura Nei distance (TN93) between analyzed taxa. Genetic distance between *Alectoris barbara* from Sardinia and Tunisia and *A. barbara* from Cyrenaica is 0.060 (6%). Standard error (in italic) is calculated on bootstrap methods with 1000 replicates.

	A. rufa	***A. chukar***	***A. barbara*** Sardinia	***A. barbara*** Tunisia	***A. barbara*** Cyrenaica
A. rufa	-	*0.021*	*0.029*	*0.029*	*0.025*
A. chukar	0.107	-	*0.023*	*0.023*	*0.025*
A. barbara Sardinia	0.162	0.118	-	*0.000*	*0.016*
A. barbara Tunisia	0.162	0.118	0.000	-	*0.016*
A. barbara Cyrenaica	0.137	0.138	**0.060**	**0.060**	-

DISCUSSION

Ghigi in 1923 was probably the last to be interested in this taxon and he considered *Alectoris barbata* as a separate species. Having had the opportunity to hold in captivity several individuals of this partridge, he emphasized, in addition to the morphological characters described above, also information on the behaviour that supported him in considering *A. barbata* a separate species (Ghigi 1920, 1921, 1923).

The high genetic distance 0.060 (6%), the morphological characteristics, and the statistically significant difference in tail length found between the samples of the two taxa analysed indicates that *Alectoris barbara barbata* should be considered as a distinct evolutionary significant unit (ESU), as for *A. graeca whitakeri*, the Sicilian endemic subspecies of *Alectoris graeca* (Randi *et al*. 2003).

However we should take into account that the partridges are sedentary species and *A. barbara barbata* is endemic to the Cyrenaica Peninsula in Northern Lybia and thus separated from the other North African populations of *A. barbara*. This isolation increased the morphologic and genetic differentiation that became so important that this taxon could be treated as a separate species, following the opinion expressed by Reichenow (1896, original description), Salvadori & Festa (1916) and Ghigi (1920, 1921, 1923).

A closer examination should be carried on with a larg-

er number of specimens, preferably with fresh material. Moreover being an ESU-separate species, ecological and demographic investigation are needed to assess the status and monitor demographic trends of *A. barbata*.

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