## Genetic analysis of a Western Reef Egret Egretta gularis from Israel

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The Western Reef Egret *Egretta gularis* is a small Palearctic heron with two subspecies, *E. g.* gularis in West Africa and E. g. schistacea in East Africa and the Middle East, east to India. It occurs in pale, dark and 'intermediate' plumage morphs. On 7 May 2015 EF, TJ and CR visited a large sewage lake a few km north of Eilat, southern Israel, a site known locally as K19. Ten species of heron had been seen there spring 2015, and an outflow at the north end of the lake, which attracts thousands of small fish, constantly has herons fishing around it. There were numerous Little Egrets Egretta garzetta and Black-crowned Night Herons Nycticorax nycticorax feeding at the outflow. EF, TJ and CR caught an intermediate morph Western Reef Egret (Plates 1–3) that had been present at the site a few days previously. Western Reef Egrets breed in Kuwait (Pope & Zogaris 2012) but otherwise are found extremely locally in the Western Palearctic with small numbers of birds seen regularly in Eilat and at some sites in Morocco, along with some occasional vagrants sighted in the Mediterranean. Identification as a Western Reef Egret was confirmed by the following features (Cramp & Simmons 1977, Dubois & Yésou 1995): it was noticeably larger in the field compared to the surrounding Little Egrets and with more aggressively dominant behaviour; the bill was long, thick and slightly decurved with a yellow lower mandible (not slender, pointed and all-dark); yellow colouration extended all the way up the tarsus (not confined to just the feet) and several of the wing feathers were tipped or completely dark grey (Plates 1–3). There were no abnormalities that might be indications of hybrid origin. Few, if any, Western Reef Egrets have previously been ringed in Israel. The bird was metal ringed (number G23994), full biometrics were taken (Table 1) and it was released on site after ringing. During the ringing process a feather was shed which was kept for DNA analysis, as no sequence from this species had been deposited in public databases.

DNA was extracted from the feather sample using the DNA Micro Kit (Qiagen, UK) with addition of dithiothreitol to 0.1 M concentration in the digestion mix and elution in 80  $\mu$ l of Qiagen buffer AE. A 1074 bp polymerase chain reaction (PCR) fragment was amplified from the cytochrome b (*cytb*) gene using primers L14993 and H16065 and conditions described in Helbig *et al* (1995). PCR was performed using BIO-X-ACT Short thermostable DNA Polymerase (Bioline, UK). The 50  $\mu$ l PCR reaction contained 28.5  $\mu$ l of ddH2O, 5  $\mu$ l of 10x Optibuffer, 1  $\mu$ l of 50 mM MgCl<sub>2</sub> solution, 3  $\mu$ l of 2 mM dNTPs, 5  $\mu$ l of 10 mM primers, 2 units of DNA Polymerase and 2  $\mu$ l of template DNA. The PCR thermal cycling programme was: 3 minutes of DNA denaturation at 95°C, 35 cycles of 95°C for 30 seconds, 45°C for 30 seconds and 72°C for 1 minute, then final extension of 72°C for 5 minutes. The PCR product was separated on a 1.5% agarose gel and isolated using the QIAquick Gel Extraction Kit (Qiagen, UK) according to the manufacturer's protocols. Gel extracted DNA was diluted to 10 ng/ml and sequenced by Source BioScience (Cambridge). The forward and reverse sequences were aligned with no discrepancy. Verified sequence was uploaded to the European Nucleotide database (accession number LN901327). This represents the

**Table 1.** Biometrics of Western Reef Egret Egretta gularis, ring number G23994, ringed at K19, near Eilat, Israel, 7May 2015.

Euring Age	Wing	Tail	Tarsus + toe	Head + bill	Bill to feather
4	287 mm	98 mm	182 mm	160 mm	93.5 mm



Figure I (above). UPGMA gene tree based on cytb sequence of Western Reef Egret Egretta gularis from Israel compared with all Egretta cytb sequences previously deposited in public databases. Ardeola bacchus was used as an outgroup. The tree demonstrates the close relationship of gularis and garzetta and their divergence from all other taxa. Sequence accession numbers of birds in this table: Ardeola bacchus KJ190952, Egretta caerulea AF193825, E. tricolor AF193824, E. garzetta K|192197 (top) and KJ190950 (lower), E. thula AF193826, E. eulophotes KJ190949 (top) and EU072995 (lower), E. sacra K[190951, E. novaehollandiae DQ780878. Statistical bootstrap support is indicated at the nodes, based on 100 replicates.

**Plates I-3 (right and below).** Western Reef Egret *Egretta gularis*, ring number G23994, ringed at K19, near Eilat, Israel, 7 May 2015.

first deposition of Western Reef Egret sequence to a public repository.

The DNA fragment encompassing the entire coding region of the mitochondrial cytb contained no premature stop codons or frameshift mutations. It was aligned to comparable sequence from other previously sequenced Ardeidae species using Nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast. cgi). Surprisingly, the sequences from Western Reef Egret and Little Egret were almost identical. There was 1073/1074 bp identity to a Little Egret with accession number KJ192197 and 1071/1074 bp identity with another Little Egret with accession number KJ190950. Both these Little Egrets (the only ones in the European Nucleotide database) were from China, where Western Reef Egret does not occur. The E. gularis and E. garzetta cytb sequences were, in contrast, divergent from all other



*Egretta* species. The closest other species was Snowy Egret *E. thula* (31 bp difference). A gene tree was compiled using CLC sequence viewer (Figure 1). Western Reef Egret and Little Egret formed a clade with 100% bootstrap support. Western and Eastern (Pacific) Reef Egrets *E. sacra* were resolved only as distant relatives. No Western Reef Egret sequence has previously been published, but these data are consistent with a report in Christidis & Boles (2008) that Little and Western Reef Egrets had yielded identical sequences in a previous study for which the data are not publicly available.

Tentatively our data suggest a very close relationship and/or ongoing or recent gene flow between Little and Western Reef Egrets. Although most authorities and major world checklists (eg Howard & Moore, IOC, Clements) treat Little and Western Reef Egrets as separate species, primarily following Payne (1979), Western Reef Egret taxonomy has swung back and forth (summarised in Turner 2010) and is not yet fully agreed or resolved. BirdLife International (2015) continues to treat them as conspecific. The uncorrected genetic *cytb* divergence between the taxa (<0.2%) is smaller than that often observed between sister species and is more typical, though not conclusive, of a conspecific relationship (Helbig *et al* 1995). Occasional interbreeding has been reported or suspected between *garzetta* and *gularis* (Dies *et al* 2001, Kayser *et al* 2000, Qninba *et al* 2011) and it is conceivable that the level of gene flow is sufficient to maintain homogeneity at morphologically neutral genetic loci while selection on genes affecting morphology maintains the structural and plumage differences between the taxa. Further study is required to determine why these morphologically divergent birds appear to retain near-identical mtDNA alleles, and more individuals of both taxa from across their ranges need to be genetically sampled.

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