Supplementary material

DNA Extraction

DNA was extracted from caudal fin clips (1mm x 2mm) using Chelex 100 resin (BioRad) following a modification of Casquet et al. (2012). Caudal fin tissue was placed into individual 1.5ml tubes containing 400µl of 5% Chelex and 40mg proteinase K. Following an overnight incubation, tubes were heated to 90°C for 10 minutes then centrifuged at ~20,000 x g for 10 minutes.

PCR Amplification and sequencing

Approximately 1200bp of mitochondrial cytochrome b was amplified using primers situated in the flanking tRNAs; cytb-glu and cytb-thr (Waters et al. 2001). PCRs contained 0.5µM each primer and 1 x MyFi Mix (Bioline) in a total volume of 10µl and were cycled in an Eppendorf Mastercycler Pro S thermocycler: 94°C for 120 s, followed by 35 cycles of 94°C for 30 s, 47°C for 30 s, 72°C for 60 s, with a final extension of 72°C for 240 s.

Two microliters of amplified DNA was visualised on a 1% agarose gel containing SYBR safe (Thermo Fisher) using a blue LED transilluminator (UVI). The remaining DNA was purified using a MEGA quick-spin total fragment DNA purification kit (iNtRON) and quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher). Purified DNA was sequenced using primer cytb-glu on an ABI 3730xl DNA Analyser), producing up to 930bp of useable sequence after editing.

Species identification

Species identification was diagnosed using BLAST searches of the NCBI GenBank database (Altschul et al. 1990), and confirmed by aligning the sequences to a large dataset containing reference sequences and subsequent Neighbour-Joining tree building. Due to very limited sequence information in GenBank for *Galaxias argenteus*, close matches to this species were found only when a shortened fragment of the unknown DNA sequences was used.

References

Casquet J, Thebaud C, Gillespie RG. 2012. Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. Mol Ecol Resour. 12(1):136-141.

Waters JM, Craw D, Youngson JH, Wallis GP. 2001. Genes meet geology: fish phylogeographic pattern reflects ancient, rather than modern, drainage connections. Evolution. 55(9):1844-1851.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol. 215(3):403-410.