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Short Communication

Identification of naturally occurring hybrids between two overexploited sciaenid species along the South African coast

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ABSTRACT

Hybridisation between fish species can play a significant role in evolutionary processes and can influence management and conservation planning, however, this phenomenon has been widely understudied, especially in marine organisms. The distribution limits of two sciaenid species (silver kob, *Argyrosomus inodorus*, and dusky kob, *A. japonicus*) partly overlap along the South African coast, where both species have undergone severe depletion due to overfishing. Following the identification of a number of possible cases of species misidentification or hybridisation (21 out of 422 individuals), nuclear and mitochondrial DNA data (12 microsatellite loci and 562 bp of the *COI* gene) were analysed to investigate the genetic composition of these individuals. Results indicated a field-based species misidentification rate of approximately 2.8% and a rate of natural hybridisation of 0.7%. Interestingly, all hybrid fish resulted from first-generation (F1) hybridisation events, which occurred exclusively between silver kob females and dusky kob males. Whether hybridisation is the result of natural events (such as secondary contact following a shift in distribution range), or anthropogenic activities (size-selective pressure due to overfishing), these findings have important implications for critical recovery and future management of these species in the wild.

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1. Introduction

Hybridisation between genetically distinct organisms can occur as the result of anthropogenic activities, such as habitat modification or individual translocation, but also as a natural phenomenon (Stronen and Paquet, 2013). Partly due to increasing sampling effort and improved technological and analytical power, it has been shown that the occurrence of natural hybrids, especially in animals as opposed to plants, has been understudied (Mallet, 2005) and is likely to play a significant role in evolutionary processes (Abbott et al., 2013; Epifanio and Nielsen, 2000). In particular, interspecific hybridisation may facilitate adaptive radiation in adaptive zones arising from changing environmental conditions (Seehausen, 2004). Thus, detection and subsequent study of hybrids in the wild has important implications for conservation and management of species (Allendorf et al., 2001).

Silver kob (*Argyrosomus inodorus*) and dusky kob (*A. japonicus*) are two large sciaenid fish species that partly co-occur along southern coasts of South Africa, from False Bay in the Western Cape province (Atlantic Ocean) to the Kei River in the Eastern Cape Province (Indian Ocean) (Griffiths, 1996b, 1997b). Silver- and dusky kob have been identified as distinct species since 1995, following an in depth study of habitat distribution, morphometrics, otolith and anatomical structure (Griffiths and Heemstra, 1995). Although silver kob seems to have entered a recovery phase due to drastic efforts to reduce quotas in the commercial linefishery (Winker et al., 2013), wild stocks of both species have been severely depleted due to overfishing (Griffiths, 1997a,c). Specifically, spawner biomass-per-recruit has been estimated at less than 5% and 12.5% of pristine values for dusky kob and silver kob, respectively (Griffiths, 1997c), which urges a prompt evaluation of the current conservation status of these species. Following the decline of wild stocks and growing local demand for these species in the seafood market, kob farming has been initiated in the emerging South African aquaculture sector (DAFF, 2012). Although this

may relieve some pressure from the harvesting of wild stocks, it also poses some concerns due to the potential negative effects of interactions between wild and farmed fish, as it has been increasingly evident in other species (Bekkevold et al., 2006; Glover et al., 2012). The monitoring and evaluation of such interactions can, however, be challenging and depend on accurate understanding of intra- and interspecific boundaries (here intended as barriers to gene flow) (e.g. Glover et al., 2011).

In the present study, a combination of nuclear and mitochondrial DNA data was used to investigate the genetic composition of a number of individuals that were identified as possible cases of species misidentification or hybridisation between kob species along the South African coast. Specifically, the hypotheses that individuals were either misidentified in the field (when field data contrasted with both nuclear and mitochondrial DNA data) or resulted from hybridisation events (when DNA data from different marker classes were in disagreement or showed signs of mixed nuclear ancestry) were tested, and discussed in the context of exploitation and monitoring of these species.

2. Materials and methods

As part of an ongoing population genetics study, a total of 422 individuals, comprising 208 silver- and 214 dusky kob specimens, were sampled over 2000 km of coastline along three South African provinces (Western Cape, Eastern Cape and KwaZulu-Natal). Following a comparison between preliminary genetic data and species identification based on field observational data, a total of 30 individuals were selected: 17 cases of potential species misidentification (field data in disagreement with genetic data), four cases of potential hybridisation events (individuals showing mixed inter-specific ancestry) and nine positive controls (no discrepancy between field and genetic data).

Nuclear DNA data were collected from the amplification of 12 microsatellite markers, following laboratory procedures as described in Archangi et al. (2009) (UBA5, UBA6, UBA40, UBA42, UBA44, UBA50, UBA851, UBA853, UBA854) and Mirimin et al. (2013) (Ajap06, Ajap14, Ajap37). Potential presence of null alleles and genotyping errors was tested with MICROCHECKER (van Oosterhout et al., 2004). Linkage disequilibrium and Hardy–Weinberg equilibrium were tested (over the whole sample set for each species independently, excluding potential hybrids and species misidentification) using ARLEQUIN 3.5 (Excoffier et al., 2005). The Bayesian approach implemented in STRUCTURE 2.3.1 (Pritchard et al., 2000) was used to infer nuclear membership proportions of individuals without prior information on sampling location and phenotype (species identification based on field observational data). For this test, analyses were carried out in five independent Monte Carlo Markov Chain (MCMC) simulations for each of 1–5 K values, using correlated allele frequencies, an admixture model, a burn-in of 100,000 and a run length of 1,000,000. A matrix of pairwise estimates of genetic distance was calculated with the aid of GENALEX 6.5 (Peakall and Smouse, 2006, 2012). This matrix was then used to construct a NeighborNet network with SPLITSTREE 4 (Huson and Bryant, 2006). The Bayesian approach implemented in NEWHYBRIDS 1.1 (Anderson and Thompson, 2002) was applied to place genotypes into distinct pure-bred or hybrid classes (first-generation (F1), second-generation (F2) or bidirectional backcrosses (BC)). Three independent MCMC simulations were executed (100,000 burn-in and up to 2,300,000 simulations) using observed population-wide allele frequencies for each species, as a prior.

Mitochondrial DNA (mtDNA) data were obtained following sequencing of a portion of the COI gene, with primers and laboratory procedures as described by Ivanova et al. (2007). Sequence data was inspected and aligned with MEGA 5.1 (Tamura et al., 2011). Species identification was carried out by comparison of COI sequence data following a BLAST search in the GenBank database.

3. Results and discussion

Genotypic data from 12 microsatellite markers and 562 bp of the COI gene were obtained from all 30 individuals (Genbank accession numbers KJ566650-KJ566679). No evidence of null alleles, genotyping errors, linkage disequilibrium or departure from expected Hardy–Weinberg proportions were detected. Bayesian clustering analyses (STRUCTURE) revealed convergence among independent runs, indicating that the simulation settings were appropriate. The number of clusters showing the highest posterior probability was $K = 3$ (or $K = 2$ after removal of squaretail kob), reflecting the three species-specific groups as shown by COI barcoding results (see below), with the exception of some individuals showing mixed ancestry (Table 1). For all nine positive controls, genetic data matched field-based species identification (Table 1). For the test cases, a total of 12 individuals resulted as species misidentifications (Table 1): including eight silver kob individuals that were erroneously identified in the field as dusky kob (one in the Western Cape and seven in the Eastern Cape) and four dusky kob individuals that were erroneously identified in the field as silver kob (one in the Western Cape and three in the Eastern Cape). This indicates a dusky/silver kob field misidentification rate of approximately 2.8% (12 out of 422). Additionally, COI barcoding data revealed that five squaretail kob (*Argyrosomus thorpei*) individuals were erroneously identified in the field as dusky kob (all caught together in a KwaZulu-Natal location) (Table 1). However, this is likely the result of an occasional mislabelling event rather than a recurrent misidentification issue.

When estimating the posterior probabilities of individual genotypes belonging to specific pure-bred or hybrid classes (NEWHYBRIDS), all specimens that were previously identified as pure-bred were confirmed, corroborating nuclear and mtDNA analyses in the current study (Table 1). Three of the four individuals that showed mixed ancestry were identified as first-generation hybrids (F1) between dusky- and silver kob, while the fourth individual was assigned to the pure-bred dusky kob class, indicating that this may not be a true hybrid (Table 1). The nuclear DNA relationship among the 30 individuals analysed in the present study is further displayed in the NeighborNet representation, where each species formed a distinct cluster in full agreement with mtDNA barcoding data, with the exception of the three hybrid fish that showed intermediate (mixed) ancestry (Fig. 1). The fourth potential hybrid individual was placed between the dusky- and squaretail kob clusters (Fig. 1), however, the hypothesis of a dusky/squaretail hybrid was rejected.

These results indicate an estimated hybridisation incidence of 0.7% (3 out of 422) between dusky- and silver kob in the wild, which is the first reported case of hybridisation for these two species. This observation was made possible due to extensive sampling coverage carried out across the distributional ranges of the species in South African waters. Together with the absence of F2 hybrids or introgression resulting from backcrossing, these findings suggest that hybridisation between dusky kob and silver kob is currently a limited phenomenon, indicating that either F1 hybrids are incapable of reproducing successfully and/or that hybridisation between these species is a rare event. Interestingly, there is emerging evidence of inter-specific hybridisation between *A. inodorus* and west coast dusky kob (*A. coronus*) taking place in the north-western limits of silver kob's distribution range (Henriques R. Pers. Comm.).