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

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Abstract

Hybridization 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 hybridization (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 hybridization of 0.7%. Interestingly, all hybrid fish resulted from first-generation (F1) hybridization events, which occurred exclusively between silver kob females and dusky kob males. Whether hybridization 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.

Keywords: hybridization, mtDNA *COI*, microsatellites, overfishing, Argyrosomus

Introduction

Hybridization 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 hybridization 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 hybridization 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 hybridization 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.

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 2,000 km of coastline along the three coastal provinces of South Africa (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 hybridization 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) (*Ajap*06, *Ajap*14, *Ajap*37). 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.

Results and Discussion

Genotypic data from 12 microsatellite markers and 562 bp of the *COI* gene were obtained from all 30 individuals. 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, reflecting the three species-specific groups as shown by COI barcoding results (see below), with the exception of some individuals showing mixed ancestry (**Tab. 1**). For all nine positive controls, genetic data matched field-based species identification (**Tab. 1**). For the test cases, a total of 12 individuals resulted as species misidentifications (**Tab. 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)

Table 1 Comparison among morphological identification (Field code), microsatellite data assignment using STRUCTURE (*K* = 2) (Cluster membership %), mtDNA COI barcoding identity (mtDNA COI identity), class with highest posterior probability (NewHybrids) and concluding remarks (Conclusion), for a total of 30 individuals. Dusky kob: *Argyrosomus japonicus*; Silver kob: *Argyrosomus inodorus*; Squaretail kob: *Argyrosomus thorpei*. *: see text; n.a.: not applicable.

Number of individuals	Field code	Cluster membership % (Dusky/Silver)	mtDNA COI identity	NewHybrids	Conclusion
4	Dusky	100/0	Dusky	Pure dusky	Positive controls confirmed
5	Silver	0/100	Silver	Pure silver	Positive controls confirmed
8	Dusky	0/100	Silver	Pure silver	Species misidentification
4	Silver	100/0	Dusky	Pure dusky	Species misidentification
5	Dusky	n.a.	Squaretail	Pure squaretail	Species misidentification
1	Dusky	53/47	Silver	F1 hybrid	First generation hybrid
1	Dusky	55/45	Silver	F1 hybrid	First generation hybrid
1	Dusky	68/32	Dusky	Pure dusky	Not a true hybrid
1	Silver	21/79	Silver	F1 hybrid	First generation hybrid

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) (**Tab. 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 (**Tab. 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 (**Tab. 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 hybridization incidence of 0.7% (3 out of 422) between dusky- and silver kob in the wild, which is the first reported case of hybridization 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 hybridization between dusky kob and silver kob is currently a limited phenomenon, indicating that either F1 hybrids are incapable of reproducing successfully or that hybridization between these species is a rare event. Interestingly, there is emerging evidence of inter-specific hybridization 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.).

Hybridization between fish species in the wild is known to result from a range of factors, spanning from direct human intervention (e.g. Castillo et al., 2008), to natural secondary contact and/or long-term introgression (e.g. Machado-Schiaffino et al., 2010). In the case of South African kob species, spawning migrations of adults is more prevalent in dusky- than silver kob, although common breeding areas may occur in the southern and south-eastern regions (Griffiths, 1996b, 1997b). Silver kob distribution is "locked" between that of dusky kob (Indian Ocean) and west coast dusky kob (*A. coronus*) (Atlantic Ocean) (Potts et al., 2010), following adaptation to colder waters compared with the other two species. The three F1 hybrid individuals identified in the present study were sampled in the Western
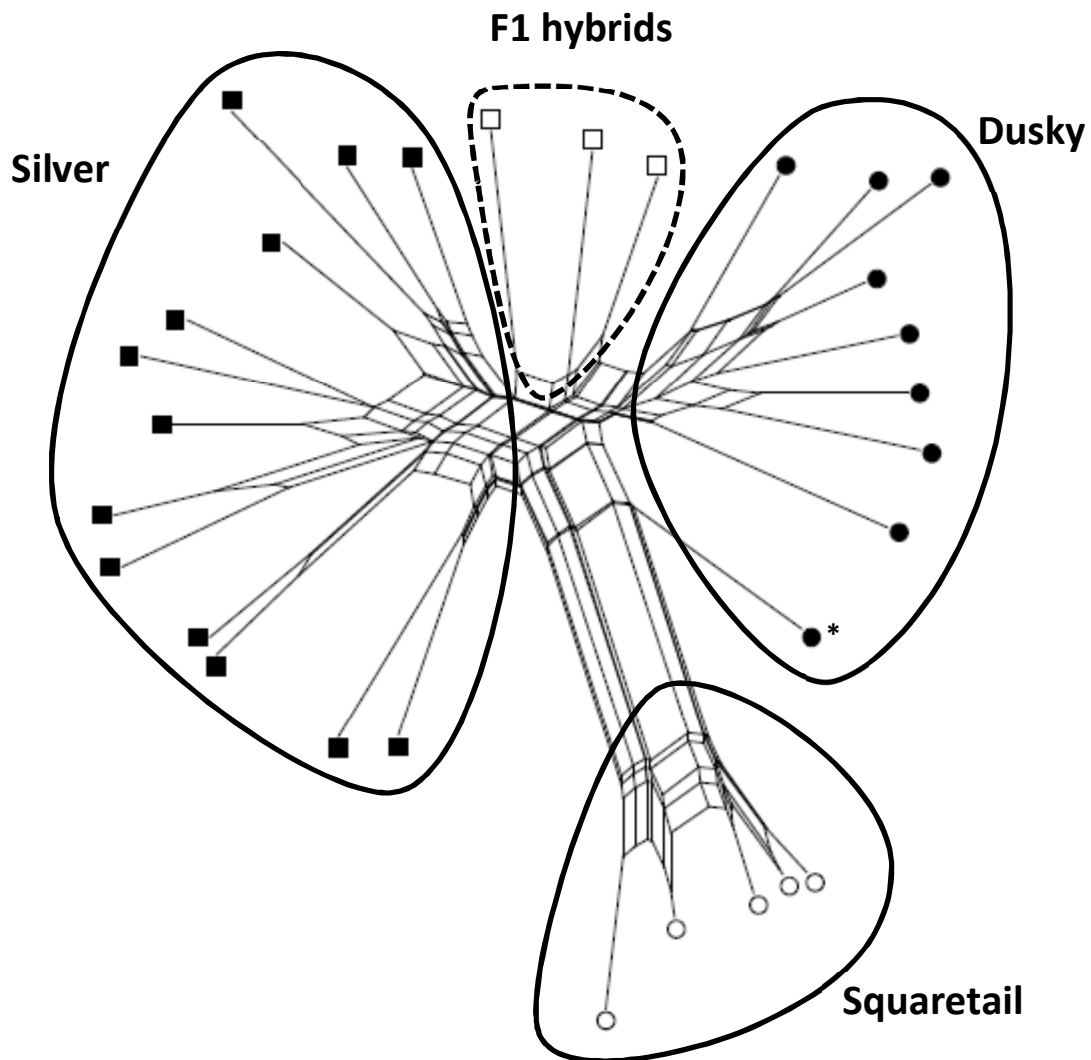
Figure 1 NeighborNet network constructed from a matrix of pairwise genetic distance (based on microsatellite genotypes) among the 30 individuals analysed in the present study, including silver kob (black filled squares), dusky kob (black filled circles), squaretail kob (white circles) and dusky/silver F1 hybrids (white squares). *: this individual proved to be not a true hybrid.

Cape (Breede River estuary) and South-Eastern Cape (Sundays River mouth and Struisbaai) in January 2013, July 2010 and May 2010, respectively, where both species co-occur. Although sex was not recorded, total body length was 300mm, 1,195mm and 510mm, respectively, indicating the ability of these individuals to reach a suitable size for sexual maturity. The ability of these species to naturally hybridize is noteworthy in view of future fish farming practices and it should also be taken into account in future conservation and management strategies of wild stocks. On the one hand, inter-specific hybrid organisms may provide enhanced traits in captivity (Chen, 2010), yet on the other hand the unintentional release of farmed hybrid fish could result in the artificial increase of hybridization frequencies in the wild. Hence, empirical studies of captive fish (in secure land-based facilities) should be encouraged in order to understand reproductive fitness of such silver-dusky kob hybrids.

All three hybrid fish identified in the present study were characterized by silver kob mtDNA haplotypes, indicating that these hybridization events occurred exclusively between silver kob females and dusky kob males, hence suggesting unidirectional hybridization (taking place exclusively between one sex of one species and the opposite sex of the other species). Given the fact that dusky kob attain sexual maturity at a much larger size than silver kob (Griffiths, 1996b, 1997b) and that drumming muscles are used during courtship in sciaenid species (Griffiths, 1997c), unidirectional hybridization could be explained by hypothesizing that large dusky kob males may occasionally out-compete silver kob males by gaining access to oestrous silver kob females. Griffiths (1996b) found that sexually mature dusky kob males (but not females) could be found throughout the distribution range of the species in South African waters and Griffiths (1997b) reported a tendency for silver kob to have a skewed sex-ratio in favour of females, which could result in increased opportunities for dusky kob males to compete with silver kob males. This could take place in areas where species-ratios are skewed such as overlapping inshore/estuarine habitat, as it is the case between silver kob and west coast dusky kob in estuaries of the south-western African coast, where the former seems to outcompete the latter (Lamberth et al., 2008). It has been hypothesized that the lower size variance at older age (> 12 years) observed in silver kob may be the result of size-selective fishing pressure (Griffiths, 1996a), however, the effect of size selection on mating behaviour and mate recognition remains untested. The present study reports findings that raise a number of life history, behavioural and evolutionary hypotheses; however, such hypotheses require further testing and support from additional empirical data. Thus, whether hybridization 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 in future management and recovery plans for sciaenid species in South African waters.

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