**Table 1** Microsatellite loci of *Dinizia excelsa*, with number of individuals sampled (*n*), number of alleles observed (*k*), observed heterozygosity ($H_O$), expected heterozygosity ($H_E$) and expected exclusion probabilities ($P_E$) calculated by CERVUS (Marshall *et al*. 1998). All sequences have been deposited in GenBank (AF143976, AF143979, AF143980, AF143982, AF143986, AF143987, AF143988)

| Locus | Repeat array | Primer sequences (5′–3′) | Annealing temp (°C) | Clone size (bp) | *n* | *k* | $H_O$ | $H_E$ | $P_E$ |
|---|---|---|---|---|---|---|---|---|---|
| DE27 | $(AAG)_8$ | GCATTTAAAAATTTAAATGTAGGG<br>GTGCAGTTTTGGATTCTTTGC | 60 | 118 | 121 | 5 | 0.54 | 0.49 | 0.23 |
| DE37 | $(AC)_{20}$ | TAGAATGTGCGCGCACGTC<br>GTGTATAACTGGTGTCACCC | 60 | 128 | 115 | 11 | 0.72 | 0.73 | 0.51 |
| DE44 | $(GT)_{13}$ | ACGCTTAAAGGCTATTGAACC<br>CAAATTTAAAAATAGATTAATTGAAAC | 60 | 144 | 119 | 9 | 0.66 | 0.64 | 0.40 |
| DE48 | $(GA)_{27}$ | AGAAGAATTAGGGAGGGACG<br>GAATAAAAGCATGCTTTATTTTTC | 60 | 143 | 106 | 31 | 0.80 | 0.94* | 0.87 |
| DE54 | $(CT)_{39}$ | GTGCAATGGGACAAAGCTTC<br>TCCCATTGCTCAAAGACTCG | 60 | 175 | 84 | 21 | 0.62 | 0.93* | 0.85 |
| DE60 | $(AAT)_7$ | CAACGCAAATAAGGCCTAACC<br>CATATATACCTGGGCTTACAG | 62 | 238 | 23 | 2 | 0.35 | 0.29 | — |
| DE64 | $(AAT)_7$ | ATTCCACTGAGGCAAATCCC<br>CCTCCGGCATTAACTCAGG | 60 | 134 | 36 | 2 | 0.03 | 0.03 | — |

*Significant excess of homozygotes ($P < 0.05$) in some of the sample populations.

for paternity analyses. Six loci that did not amplify reliably but may be useful with different primers are (repeat array followed by GenBank Accession no.): $(GA)_{13}$ (AF143977); $(TG)_{13}$ (AF143978); $(CT)_{19}$ (AF143981); $(TG)_{11}$ (AF143983); $(GA)_{24}$ (AF143984); and $(CT)_{23}(AT)_{12}$ (AF143985).

The five loci used for paternity yielded 77 alleles with a mean of 15 alleles per locus. Observed and expected heterozygosities ranged from 0.49 to 0.94. Hardy–Weinberg equilibrium was tested with Fisher's exact test of GENEPOP version 3.1d (Raymond & Rousset 1995). A significant excess of homozygotes was observed in three populations for DE48 and in five populations for DE54 (Table 1), presumably the result of null alleles. The expected exclusion probabilities for single loci ranged from 0.23 to 0.87 with a multilocus expectation of > 0.995 (Marshall *et al*. 1998). Together, these loci provided enough variation to resolve paternity for a large portion of the *D. excelsa* seed population.

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## Amplifying dolphin mitochondrial DNA from faecal plumes

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Molecular studies are increasingly used to support cetacean conservation and management (Hoelzel 1994). Biopsy-darting

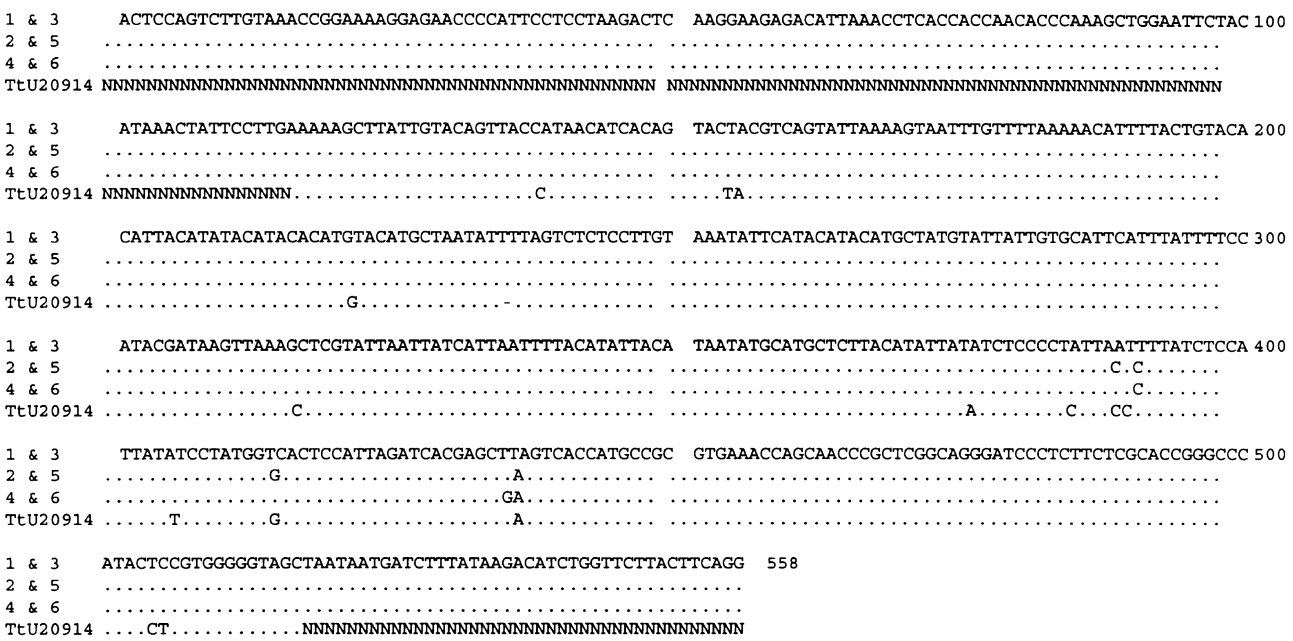


**Fig. 1** Aligned control region sequences of DNA extracted from five *Tursiops truncatus* faecal samples (1 through 5; GenBank Accession no. AF155160–AF155162), one *T. truncatus* skin sample (6), and a 400-bp *T. truncatus* sequence from GenBank.

(e.g. Lambertsen 1987) and collecting sloughed skin (e.g. Amos *et al*. 1992) have provided DNA from several species, but these methods are often impractical. An alternative is to extract DNA from faeces, as previously applied to terrestrial (e.g. Gerloff *et al*. 1995) and semiaquatic (Reed *et al*. 1997) mammals, and to floating dugong faeces (Tikel *et al*. 1996). Until now, however, the diffuse form of cetacean 'faecal plumes' has prevented their collection and genetic analysis. Here, we demonstrate the feasibility of collecting faeces, extracting total DNA, and reliably amplifying mitochondrial DNA (mtDNA) markers from free-ranging dolphins.

Five faecal samples were collected from bottlenose dolphins (*Tursiops truncatus*) in the Bahamas (26°0′N 77°25′W). Upon observing defaecation, snorkelers collected sinking faeces in 100 mL plastic vials. Samples were immediately preserved in 20% DMSO/5 M NaCl (Amos & Hoelzel 1991), and frozen at –20 °C within 10 h.

Faeces were thawed immediately preceding extraction (total storage time 157–227 days). Cross-contamination was avoided by using disposable plasticware, and by cleaning metal instruments with ethanol, and flaming. One millilitre of faeces was suspended in 2 mL of 2× CTAB buffer (100 mM Tris-HCl, pH 8, 1.4 M NaCl, 20 mM EDTA, 2% CTAB) (Milligan 1992), and mixed by rotation. Debris was cleared by centrifugation, and 2 mL of lysate transferred to a 2-mL microcentrifuge tube. Following high-speed centrifugation, 1.5 mL of cleared suspension was extracted twice with 0.5 mL of chloroform, and DNA precipitated by adding 0.67 mL of isopropanol.

DNA pellets were dissolved in 1.8 mL of guanidine thiocyanate (GITC) buffer (5 M GITC, 0.1 M Tris-HCl, pH 6.4) at ambient temperature overnight. Three grammes of diatomaceous earth particles (Sigma D-5384) were washed, three times, in 50 mL of molecular-grade water, and centrifuged. Pelleted diatoms were then suspended in 12 mL of water and vortexed to create a slurry. One hundred microlitres of diatomaceous slurry was added to the microfuge tube to recover the DNA. Diatoms were pelleted, resuspended in 600 µL of GITC buffer, transferred to the insert of a 10-µm mesh VectaSpin microtube (Whatman, no. 6838 0002) and centrifuged to remove buffer. The diatoms, and DNA, were washed twice with 700 µL of ethanol, then incubated at 55 °C for 30 min to evaporate residual traces of ethanol. DNA was eluted in 130 µL of TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), incubated at 55 °C for 30 min, centrifuged to pellet diatom traces, and 100 µL was transferred to a new 0.2-mL tube.

The origin and quality of extracted DNA was determined by polymerase chain reaction (PCR) amplification of 558 bp of the 5′ section of the mtDNA control region, using primers L15926* and H16498 (Eggert *et al*. 1998). PCR reactions were carried out in a 50-µL volume containing 10 µL of DNA. Extraction blanks, positive and negative controls were included in all PCR reactions to detect contamination. Products were detected and quantified on ethidium-stained 1% agarose gels. The desired fragment was successfully amplified from the DNA of all five faecal samples.

To confirm that amplified mtDNA fragments originated from *T. truncatus*, both strands of purified (QIAquick PCR purification kit [Qiagen]) PCR products were sequenced using BigDye sequencing kits (PE Biosystems), and detected on an ABI 377 automated DNA sequencer. Each faecal sample was amplified and sequenced twice. These sequences were aligned to a published *T. truncatus* mtDNA sequence (GenBank), and to the sequence from a *T. truncatus* skin sample obtained in the Bahamas (Fig. 1). All five faecal sequences showed a high degree of similarity to the published sequence (15

substitution sites; 14 transitions), and to the sequence from dolphin skin (five substitution sites; five transitions). This indicates that the amplified mtDNA sequences originated from dolphin DNA, and not from contaminating sources of faecal (e.g. prey) DNA.

Low quantities of target DNA can cause complications when using faecal-derived DNA in nuclear genotyping (Taberlet & Waits 1998). However, our mtDNA analyses produced reliable results from the sequence of a single fragment, suggesting that faeces may represent a useful supplement to conventional sources of DNA for future studies of cetacean population genetics.

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