Primers, PCR conditions, purification of PCR products and sequencing reaction conditions

			PCR conditions				
gene region	primer	sequence (5' to 3')	Denaturation	Annealing	Extension	cycles	Reference
barcode	LCO, F	GGTCAACAAATCATAAAGATATTGG					N. Wahlberg
(1st half *CoI*)	Lep 3.1, R	AAATTTTAATTCCTGTTGGTACAGC	94ºC for 30s	47ºC for 30s	72ºC for 1.5min	35	Dasmahapatra et al., in review
	Nancy, R	CCTGGTAAAATTAAAATATAAACTTC					N. Wahlberg
	K698=Rush, F	TACAATTTATCGCCTAAACTTCAGCC					Silva-Brandão et al. (2005), Brower et al. (2006)
	K699, R	WGGGGGGTAAACTGTTCATCC					N. Wahlberg
	Ron, F	GGATCACCTGATATAGCATTCCC	95 for 1 min	45 for 1 min	72 for 1.5 min.	33	Brower et al. (2006)
	Jane, R	TAAAATTACTCCTGTTAATCCTCC					Brower et al. (2006)
	Wyman, R	GYTGAGCTCAWACAATAAATCCTA					Brower et al. (2006)
CoI	Jerry, F	CAACAYTTATTTTGATTTTTTGG	94ºC for 30s	47ºC for 30s	72ºC for 1.5min	35	Simon et al. (1994), Mallarino et al. (2005)
(2nd half)	Pat, R	ATCCATTACATATAATCTGCCATA					Simon et al. (1994), Mallarino et al. (2005)
	Rudy, F	GAAGTTTATATTTTAATTTTACCGGG	95 for 1 min	45 for 1 min	72 for 1.5 min.	33	Brower and Jeansonne (2004)
CoII	Geoith, F	TAGGWTTAGCWGGAATACC	94ºC for 45s	55ºC for 45s	72ºC for 1.5min	35	Dasmahapatra, unpub.
	Evaith, R	GAGACCAATACTTGCTTTCAGACATCT					
	GeorgeIII, F	TAGGTITAGCIGGAATACCTCG	95 for 1 min	45 for 1 min	72 for 1.5 min.	33	Brower and Jeansonne (2004)
	Imelda, R	CATTAGAAGTAATTGCTAATTTACTA					Brower et al. (2006)
	Eva, R	GAGACCATTACTTGCTTTCAGTCATCT					Brower and Jeansonne (2004)
	Strom, F internal	TAATTTGAACTATYTTACCIG					Brower and Jeansonne (2004)
EF1a	Ef-M44-1, F	CTGAGCGYGARCGTGGTAT	94ºC for 1min	55ºC for 1min	72ºC for 1.5min	35	Cho et al. (1995), Mallarino et al. (2005)
	EFrcM4, R	ACAGCVACKGTYTGYCTCATRTC					Cho et al. (1995), Mallarino et al. (2005)
	Ef1a-257F, F internal	TATCACTATTGACATCGC					
	40 (M3 of Cho in part), F	GTCGTSATYGGWCACGTMGATT	94 for 1 min	62 for 1 min.	72 for 1.5 min.	37	Cho et al. (1995)
	Gennifer, R	CGCACGGCAAAACGACCGAGRGG					Brower et al. (2006)
	52.6 (reM52.6 of Cho), R	GCYTCGTGGTGCATYTCSAC					Cho et al. (1995)
	AL (M46-1 of Cho), F	GAGGAAATYAARAAGGAAG					Cho et al. (1995)

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References

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PCR were performed in 20 µl volume, with 2 µl template DNA, 0.4 U Bioline Taq Polymerase, 2 µl 10X buffer, 2 mM $MgCl_2$, 0.2 µM primer F, 0.2 µM primer R, 0.25 mM dNTP and ddH_2O.

PCR products were purified by adding 1U of shrimp alkaline phosphatase (SAP) and 1U of exonuclease I diluted in 1.5µl of USB SAP dilution buffer. Samples were incubated at 37ºC for 40min, and the enzymes were inactivated at 80ºC for 15min. Sequencing was carried out in both directions. One microliter of the purified PCR product was used as a template in a 10µl cycle sequence reaction containing 1µl Big Dye 3.1 (Applied Biosystems), 2µl 5X sequencing buffer (Applied Biosystems), 0.32 µM primer and ddH_2O.