The following supplement accompanies the article

An integrated mark-recapture and genetic approach to estimate the population size of white shark in South Africa

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Photographic capture-mark-recapture-technique

Table S1 Sampling effort of each month: column 1 = month of sampling or capture occasion, column 2 = number of sampling days at each month, column 3 = number of photographic identifications collected (excluding multiple photographs of the same shark collected on the same day), and column 4 = total number of identified sharks (n_i). The last two columns indicate, for identified sharks, how many were newly identified (u_i) and how many were sharks previously included in the database (m_i).

SAMPLING EFFORT			IDENTIFIED SHARKS		
Capture Occasions (i)	**Sampling Day**	**Photo ID**	**Captured sharks (n_i)**	**Newly Identified Sharks (u_i)**	**Monthly Recaptured Sharks (m_i)**
2009 04	17	40	28	28	0
2009 05	21	57	41	35	6
2009 06	16	87	70	58	12
2009 07	10	46	34	21	13
2010 02	5	23	18	17	1
2010 03	19	69	43	24	19
2010 04	14	65	42	22	20
2010 05	13	79	57	32	25
2010 06	8	35	28	10	18
2010 07	10	27	21	7	14
2010 08	8	34	28	12	16
2010 09	13	94	61	31	30
2010 10	14	39	32	11	21
2010 11	12	39	31	14	17
2010 12	12	37	21	4	17
2011 01	13	29	20	9	11
2011 02	11	57	36	17	19
2011 03	11	73	38	7	31
2011 04	11	49	36	3	33
2011 05	10	70	55	22	33
2011 06	9	60	51	9	42
2011 07	12	66	34	9	25
2011 08	5	12	10	3	7
2011 09	7	29	27	8	19
2011 10	10	58	39	5	34
2011 11	2	13	13	2	11
2011 12	5	17	14	6	8
TOT	**298**	**1304**	**928**	**426**	**502**

The mean estimated total length of the study animals (N = 426), based on size of first capture was 3.14 m, with a range of 1.30–5.50 m TL and a 3.20 m median (Fig. S1a). The sex ratio (males:females) was 1:1.09 (N = 313) (Fig. S1c), which was not significantly different from equality according to the exact binomial test (p = 0.20). There was also no evidence of any relationship between sex and size (Fig. S1b).

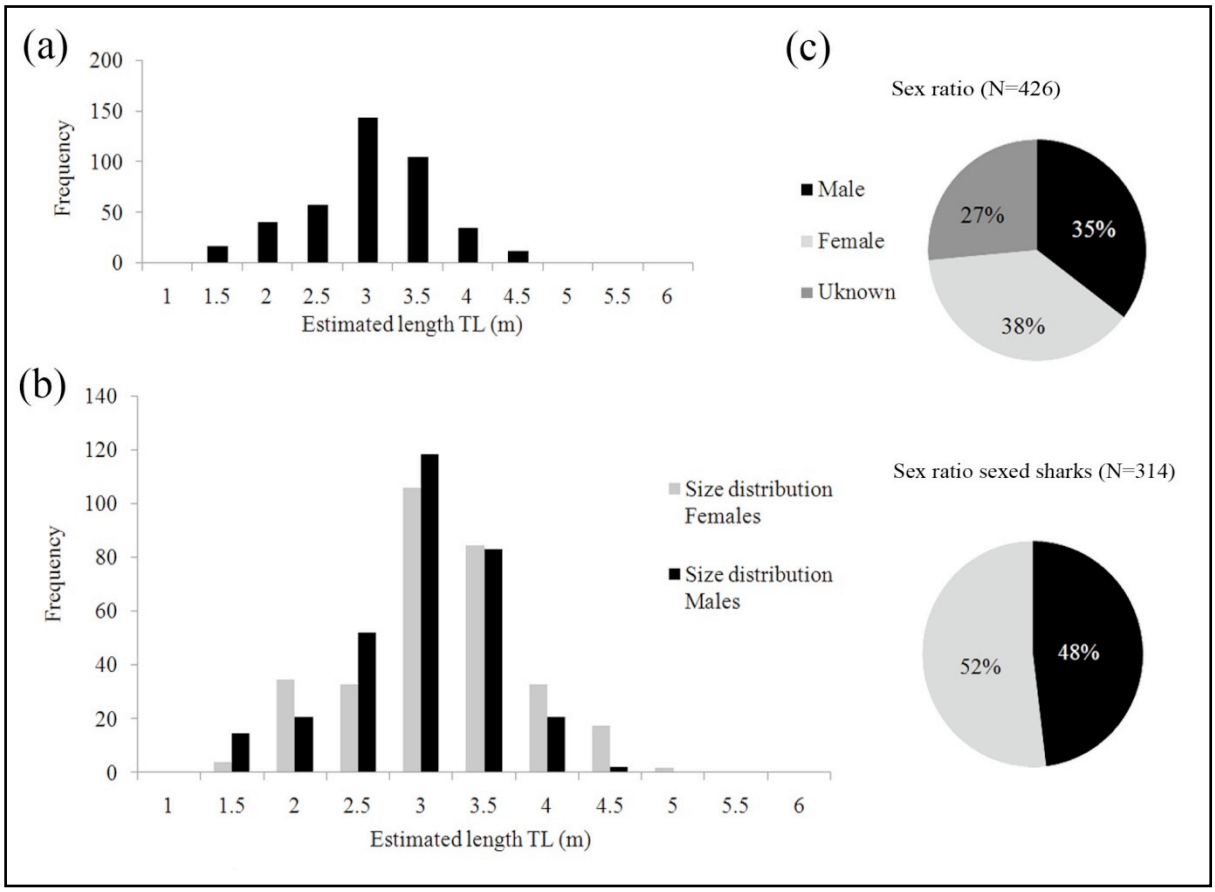

Figure S1 (a) Size distribution of 426 white sharks at Gansbaai, South Africa; (b) Length distribution per sex based on 315 of these animals that could be sexed (female: grey, male: black) and (c) sex ratio (female: grey, male: black, unknown: dark gray).

The total number of sharks identified during each season in 2010 and 2011 were normalized (based on the sampling occasions in each season). The results were compared to evaluate seasonal variation in the sharks' local abundance (Fig. S2). Seasonality was evident with the highest abundance during winter (May-July) and lowest abundance occurring during summer (November-January). Finally the counts of re-capture events during adjacent weeks indicated that white sharks, on average remain in the study area for a week, or a maximum of two weeks (Andreotti et al. unpublished data), minimizing the possibility of pseudo replications of sharks across adjacent months.

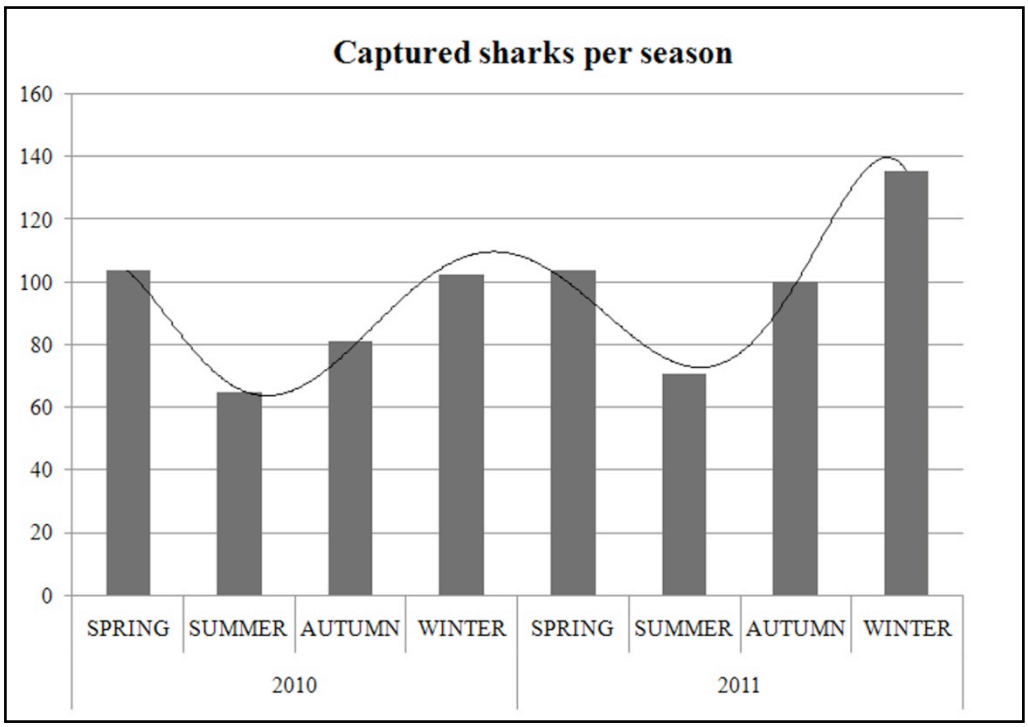

Figure S2 Seasonal occurrence of white sharks at the Dyer Island Nature Reserve (Gansbaai, South Africa). The values are normalized per sampling occasion to compare months in which the sampling effort differs due to the weather conditions.

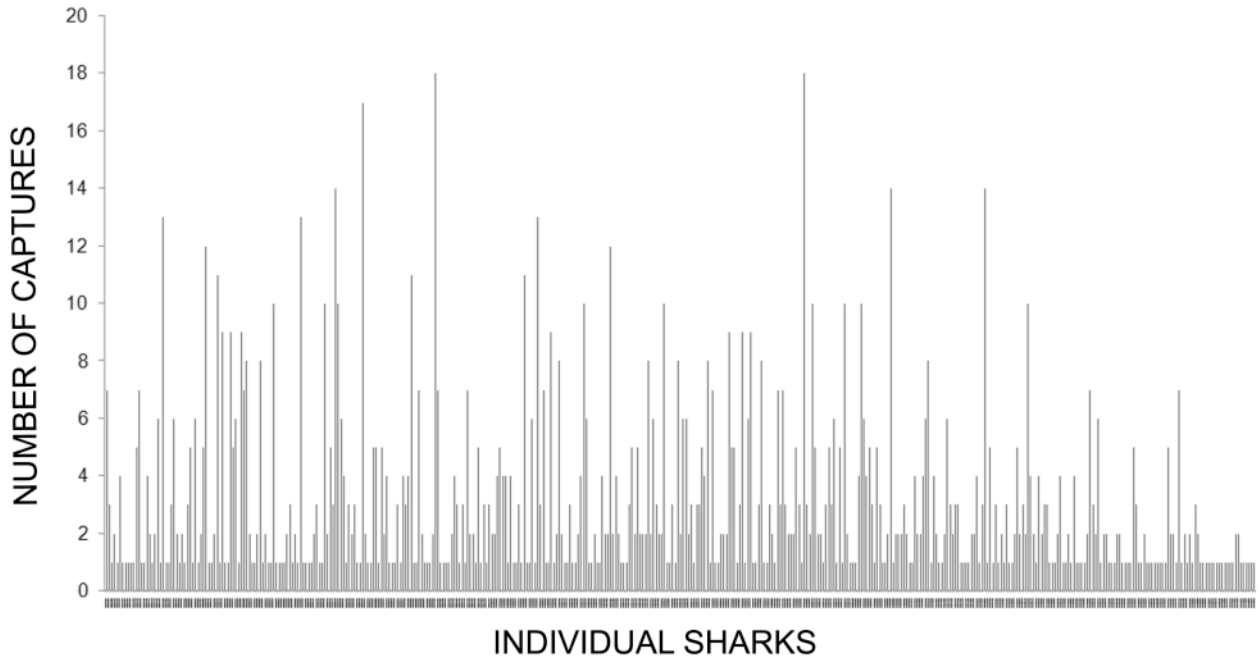

Figure S3 Captures of each individual shark (N = 426) included in the dataset. The individuals are organized by their first date of capture (left to right, from 2009 to 2011).

Genetic Technique

Table S2: Microsatellite primer details, multiplex panels and annealing temperature.

MULTIPLEX PANEL	LOCUS	PRIMERS	Repeat	Annealing Temperature (°C)	Dyes	Range
A				**50.0**		
	CCa1419	ATTATCGCATTGGGGGATTT GCAGCAGTCACTCTTGGGTA	$[TG]_{13}$		6-FAM	150-190
	CCa83	CCTTAAAAGCACAGAACAAAGATAAA GGGGATTTACAGAGAGCATCC	$[TAGA]_{16}$		VIC	185-220
	CCa1536	ACTCCGGATTGGTGCTATTG TTGGTCCTCCTTTGCTGAAT	$[TG]_{19}$		NED	230-270
Simplex				**55.0**		
	CCa1273	TGTTTTTGCCTTTTATCCTTGAA CCAGAAACCCACCCAAGTAA	$[TG]_{10}$		PET	165-195
B				**60.0**		
	Ccar1	GCAGAGGTTGGGAAAGAGTT GCTATTCCAGTGACACTCTCC	$[AC]_{22}$		6-FAM	155-170
	CCa711	GATGATTTTGCATGTCACTTTGA CTTTGCCTAATATTATTGAGAGAT	$[CA]_{19}$		VIC	165-195
	CCa1072	CCCTGTGTTCTTGCACAATG CCATTGAAGCCCTGTGAAGT	$[AC]_{26}$		NED	190-240
	Ccar6.27x	GAGCATGTGTGGGAGCGAAAG TGGGACGATTCTGCCATTCTCTC	NA		PET	165-186
C				**60.6**		
	CCa1466	ATGTGTGCAAGCAAGTCTGC GCATAACACCCCCACAGAAG	$[TG]_9[TG]_{11}$		6-FAM	230-250
	CCa1276	CCTAGCATTATGGTCAACATCAG GGTCACTTTCAACTTGAGCAAA	$[TG]_{21}$		VIC	155-195
	CCa1226	CTCTGGTTTCCTCCCAAGGT CAGGAGATGGGCACTACACA	$[TG]_{12}$		PET	150-170
D				**63.2**		
	Iox10	AGGAAATTAGGTGGGGAGGCAG GCCAAATAGATTCTGTCCTTGACCC	$[GA]_{18}$		6-FAM	110-130
	Ccar9	AATGGGTTGTGATGGGAGTTT CAAGTGGAAGTCAAGCAGGTT	$[TG]_{23}$		NED	200-247
	Ccar13	GCTGAGTGCTGGCTGACCT TATCCAGTTACCATCTCCAAAAA	$[TG]_4TT[TG]_9TT[TG]_3TTTT[TG]_{23}$		PET	265-300

Table S3: Estimates of F_{IS} (Weir & Cockerham 1984), and heterozygote deficit test p / heterozygote excess test p (within parentheses) per locus and sample calculated with FSTAT (based on 1000 randomisations). Values in bold p ≤ 0.05

	Sites				
Locus	**Algoa Bay (N =9)**	**False Bay (N = 11)**	**Gansbaai (N = 167)**	**Struisbaai (N = 18)**	**Mossel Bay (N = 28)**
Cca1419	-0.800 (1.000/**0.013**)	-0.833 (1.000/**0.005**)	-0.933 (1.000/**0.001**)	-0.744 (1.000/**0.001**)	-0.931 (1.000/**0.001**)
Cca83	-0.358 (1.000/**0.044**)	-0.034 (0.709/0.582)	0.012 (0.415/0.678)	-0.268 (0.996/**0.040**)	0.043 (0.381/0.794)
Cca1536	-0.255 (0.989/0.176)	0.003 (0.989/0.176)	-0.053 (0.942/0.086)	-0.214 (0.996/0.055)	0.048 (0.361/0.809)
Cca1273	0.351 (0.346/0.974)	0.130 (0.603/0.893)	0.054 (0.307/0.808)	-0.417 (1.000/0.114)	-0.180 (0.919/0.284)
Ccar1	-0.231 (0.935/0.264)	-0.299 (0.976/0.122)	-0.067 (0.939/0.090)	-0.256 (0.984/0.080)	0.135 (0.178/0.903)
Cca711	0.111 (0.412/0.858)	-0.143 (0.929/0.276)	-0.050 (0.902/0.151)	-0.067 (0.786/0.446)	0.091 (0.249/0.879)
Cca1072	0.175 (0.283/0.914)	0.158 (0.196/0.944)	0.155 (**0.001**/1.000)	0.069 (0.354/0.834)	0.076 (0.276/0.865)
Ccar6_27x	-0.079 (0.736/0.548)	-0.053 (1.000/0.962)	-0.020 (0.657/0.421)	-0.140 (0.852/0.340)	-0.254 (0.990/0.056)
Cca1466	-0.143 (1.000/0.838)	-0.429 (1.000/0.249)	-0.276 (1.000/**0.001**)	-0.133 (1.000/0.709)	-0.292 (1.000/0.099)
Cca1276	0.082 (0.429/0.861)	-0.209 (1.000/0.143)	-0.067 (0.992/**0.015**)	0.043 (0.419/0.811)	-0.029 (0.749/0.494)
Cca1226	-0.012 (1.000/0.479)	0.000 (0.751/0.772)	0.094 (0.093/0.961)	0.019 (0.508/0.708)	0.112 (0.288/0.881)
Iox10	-0.012 (0.649/0.664)	0.237 (0.249/0.919)	0.003 (0.525/0.556)	-0.106 (0.867/0.350)	0.017 (0.519/0.661)
Ccar9	0.067 (0.513/0.851)	-0.123 (0.946/0.368)	0.098 (0.004/**0.997**)	0.154 (0.133/0.967)	-0.059 (0.881/0.320)
Ccar13	0.056 (0.540/0.789)	-0.012 (0.698/0.643)	-0.061 (0.926/0.106)	-0.126 (0.934/0.302)	-0.071 (0.800/0.388)

Table S4: Estimates of heterozygote deficit ($D = (H_O–H_E)/H_E$), and Hardy-Weinberg equilibrium tests: heterozygote deficit test p / heterozygote excess test p (within parentheses) per locus and sample, calculated with GENEPOP (based on 1000 iterations). Values in bold p ≤ 0.05

	Sites				
Locus	**Algoa Bay (N =9)**	**False Bay (N = 11)**	**Gansbaai (N = 167)**	**Struisbaai (N = 18)**	**Mossel Bay (N = 28)**
Cca1419	0.719 (1.000/**0.009**)	0.763 (1.000/**0.004**)	0.927 (1.000/**0.000**)	0.707 (1.000/**0.000**)	0.899 (1.000/**0.000**)
Cca83	0.330 (1.000/**0.044**)	0.033 (0.468/0.567)	-0.012 (0.105/0.895)	0.258 (0.995/**0.031**)	-0.042 (0.307/0.705)
Cca1536	0.236 (0.991/0.160)	-0.026 (0.584/0.670)	0.053 (0.937/0.064)	0.207 (0.993/0.053)	-0.047 (0.299/0.709)
Cca1273	-0.338 (0.344/0.969)	-0.125 (0.604/0.891)	-0.054 (0.302/0.806)	0.400 (1.000/0.110)	0.176 (0.914/0.292)
Ccar1	0.214 (0.940/0.186)	0.280 (0.891/0.116)	0.067 (0.982/**0.018**)	0.247 (0.983/0.071)	-0.133 (0.247/0.759)
Cca711	-0.133 (0.410/0.820)	0.070 (0.930/0.286)	0.042 (0.885/0.116)	-0.042 (0.538/0.472)	-0.130 (0.258/0.766)
Cca1072	-0.167 (0.227/0.928)	-0.152 (0.114/0.906)	-0.155 (**0.000**/1.000)	-0.068 (0.190/0.827)	-0.075 (0.072/0.930)
Ccar6_27x	0.074 (0.745/0.532)	0.050 (1.000/0.954)	0.020 (0.527/0.479)	0.136 (0.870/0.230)	0.248 (0.993/**0.046**)
Cca1466	0.133 (1.000/0.823)	0.400 (1.000/0.245)	0.275 (1.000/**0.000**)	0.129 (1.000/0.725)	0.284 (1.000/0.090)
Cca1276	-0.078 (0.147/0.905)	0.197 (1.000/0.129)	0.066 (0.988/**0.012**)	-0.042 (0.380/0.710)	0.029 (0.808/0.324)
Cca1226	0.197 (1.000/0.467)	0.000 (0.756/0.766)	-0.094 (0.146/0.859)	-0.019 (0.511/0.720)	-0.110 (0.322/0.843)
Iox10	0.011 (0.363/0.659)	-0.228 (0.238/0.892)	-0.003 (0.583/0.419)	0.102 (0.720/0.331)	-0.017 (0.665/0.388)
Ccar9	-0.063 (0.526/0.792)	0.115 (0.961/0.323)	-0.097 (**0.000**/1.000)	-0.149 (0.028/0.977)	0.058 (0.422/0.590)
Ccar13	0.125 (0.558/0.542)	0.184 (0.561/0.664)	0.170 (0.491/0.509)	0.064 (0.928/0.247)	0.075 (0.800/0.304)

Table S5: Proportion of tests that generated an F_{IT} value above the expected (p) per locus and for the overall sample calculated with FSTAT (based on 1000 randomisations). Values in bold p ≤ 0.05

Locus	Overall
Cca1419	0.999
Cca83	0.663
Cca1536	0.925
Cca1273	0.527
Ccar1	0.979
Cca711	0.851
Cca1072	**0.001**
Ccar6_27x	0.826
Cca1466	0.999
Cca1276	0.996
Cca1226	0.073
Iox10	0.412
Ccar9	**0.010**
Ccar13	0.955
All loci	0.999

Table S6: Heterozygote deficit estimates (*D*), and Hardy-Weinberg equilibrium tests presented by χ^2, degrees of freedom (10) and corresponding p per locus and for the overall sample, as calculated with GENEPOP (based on 1000 iterations). Values in bold p ≤ 0.05

Locus	*D*	$\chi^2_{[10]}$	p
Cca1419	0.901	∞	**0.000**
Cca83	0.018	12.185	0.272
Cca1536	0.052	7.123	0.714
Cca1273	0.000	8.681	0.562
Ccar1	0.074	11.965	0.287
Cca711	0.011	3.229	0.976
Cca1072	-0.139	∞	**0.000**
Ccar6_27x	0.054	2.602	0.989
Cca1466	0.274	∞	**0.000**
Cca1276	0.057	5.173	0.879
Cca1226	-0.071	6.911	0.734
Iox10	-0.006	7.477	0.680
Ccar9	-0.070	18.958	**0.041**
Ccar13	0.155	14.697	0.144

Results of the different CN_e values obtained varying P_{crit}

Following Waples & Do (2010), given the large sample size (>100) and the 14 nuclear markers utilized in this study, we could obtain acceptable estimates of CN_e with an optimal allele frequency exclusion criterion P_{crit} of 0.01 (Waples & Do 2010). When analyzing separate cohorts the reduced sample size allowed to obtain reliable estimates at P_{crit} = 0.02. The other CNe values obtained with the software Ne estimator (Do et al. 2014) with different P_{crit} are indicated in Table S7 (see also Figure 3 in the main article):

Table S7 Different CN_e values obtained with the software N_e estimator (Do et al. 2014) by varying P_{crit} from 0+ to 0.05 on the complete dataset (n = 233), the Gansbaai dataset alone (n = 167) and in the two cohorts of juveniles (n = 66) and adults (n = 33). The values presented in the main article are highlighted in bold.

GROUPS	P_{crit}	0.05	0.04	0.03	0.02	0.01	0+
ALL SHARKS	Estimated CNe	374	350	314	**333**	**331**	381
	95% CIs for Ne^	252-655	247-561	233-460	**247-487**	**251-467**	287-549
GANSBAAI	Estimated CNe	445	490	369	**351**	424	436
	95% CIs for Ne^	260 - 1230	280 - 1505	239-728	**234-642**	278-813	293-798
JUVENILES ≥2.5m;≤3m	Estimated CNe	273	364	296	**303**	523	552
	95% CIs for Ne^	130-10238	158-∞	147-3566	**152-3048**	202-∞	210-∞
ADULTS F≥4.5m;M≥3.8m	Estimated CNe	∞	286	435	**200**	408	408
	95% CIs for Ne^	96-∞	70-∞	84-∞	**68-∞**	89-∞	89-∞

Before calculating N_b/N_e ratio with one white sharks age group, we adjusted the N_b values, by applying the formula (8) from (Waples et al. 2014):

$$Nb_{(Adj)} = \frac{Nb}{1.26 - 0.323 \times (Nb \div Ne)}$$

$N_b = 303$, $N_{b(Adj)} = 293$

Validation of DARWIN for white sharks photographic identifications

Materials and methods

DARWIN [Version 2.22 – (Stanley 1995)] allows for comparison ("Fin Matching") of traces (lines automatically drawn around the edge of the dorsal fin by the function "Fin Trace") to match a new fin photograph with one existing in the database. "Fin Matching" ranks all database photographs by probability of match; if it succeeds to match a new trace precisely with an existing fin trace in the database, that match will be ranked in the first position (Rank = 1).

To validate the accuracy of DARWIN, 426 individual high quality white shark photo identifications (Andreotti et al. 2014) derived from 4398 pictures, were used to test-match 122 additional images from 53 unique sharks. To optimize matching success, the edges of the dorsal fin traces were manually adjusted when "Fin Trace" failed to accurately detect the notches in the fin. The trial comprised matching the 426 traced images with: (1) 50 duplicate photographs copied from the database as the control (the same photographs were expected to match 100% with themselves, Rank = 1) (2) 50 random white sharks photographs taken from the entire collection of 4398 pictures (3) 72 photographs of three repeatedly sampled white sharks. The validation of DARWIN was based on the null hypothesis that the rank of the correct matching image from the database would be equal to one. To be less stringent, we also allowed a match within the top 20 ranked positions. A paired t-test was used to quantify how much the means of the DARWIN result (e.g. rank obtained) against the correct result (e.g. Rank = 1) differ among each other. Likewise, to allow for minor failure, we also allowed for a hypothesized difference of 20, compared to the real mean difference between the two.

Results

Validation program DARWIN for white sharks photographic identifications

"Fin Trace" failed to automatically draw an accurate edge around the dorsal fin and manual optimization was needed in each instance (Fig. S4).

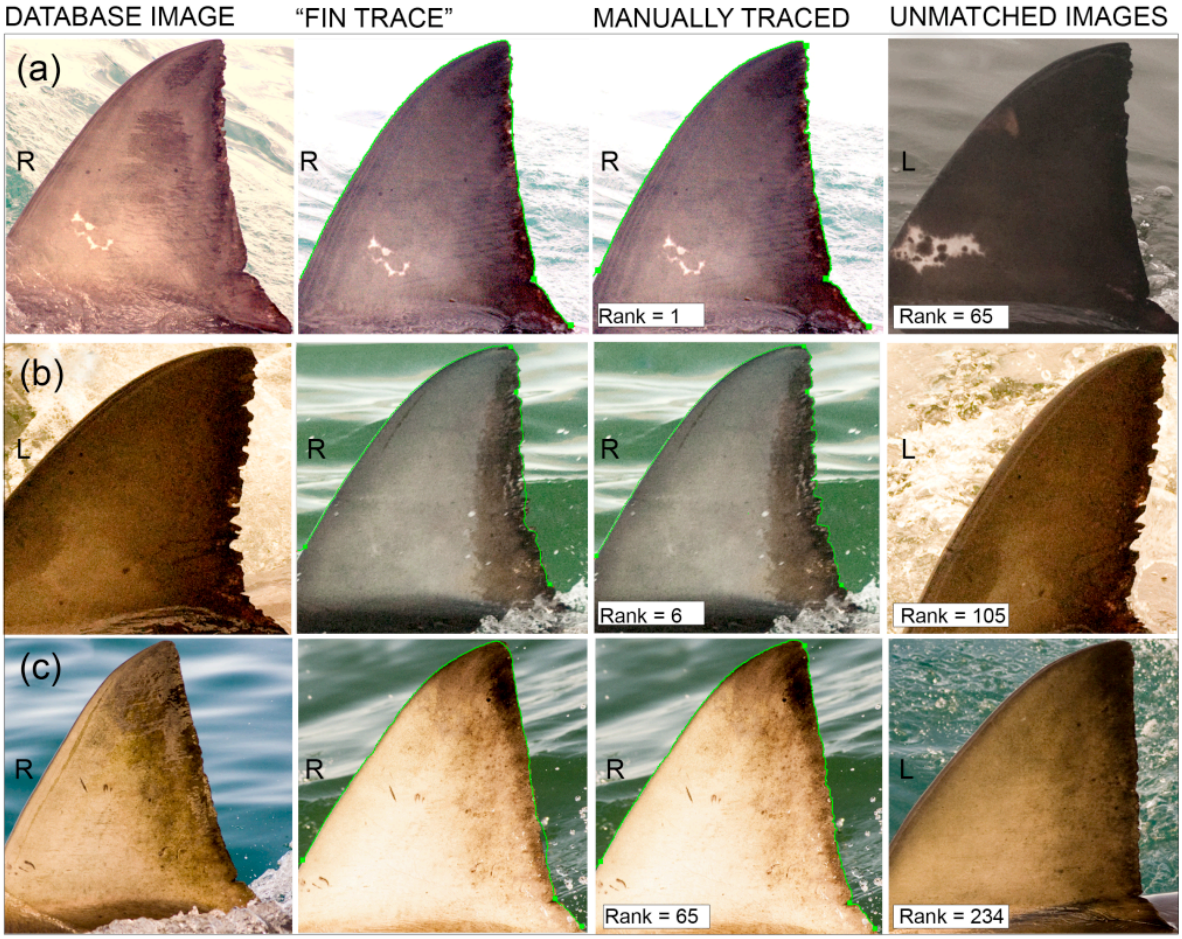

Figure S4 Example of photo identification for three different great white sharks (one row for each shark). Due to DARWIN's inherent prerequisite to work with fins from the left side only, some of the photographs were rotated horizontally (the true side of the fin is indicated as R = right, L = left, on the image). Procedures flow from the left: (1) image from the database; (2) image to be matched showing the line traced automatically by the software "Fin Trace"; (3) images with the line manually re-traced to allow for better comparison within "Fin Matching"; (4) examples of an image that could not be matched by the software. Row (a) is an example of a matched image (Ranked=1); Row (b) Example of a correct match found in the first 20 ranked images (Ranked=6); Row (c) example of an unmatched image (Ranked=65). The Rank assigned by the software to the correctly matched image is indicated on the photograph.

After adjusting the edge, 86% of the control pictures (e.g. photographs copied from the database) were correctly matched by DARWIN (Rank = 1). In the validation trial DARWIN correctly matched 20% of the photographs from the collection and an additional 20% could be found in the first 20 ranked images. The remaining 60% ranked lower than 20, with some matches as low as position 234 (Fig. S4). The trial conducted with photographs of the same three re-sighted individuals showed similar results (Fig. S5). The average difference (95% confidence) between first rank and the software's match is 66.34. The paired t-test rejected the null hypothesis that DARWIN can successfully match a white shark dorsal fin image in the upper range of up to 20 ranked images (t-stat=5.73, $p = 3.72 \times 10^{-8}$).

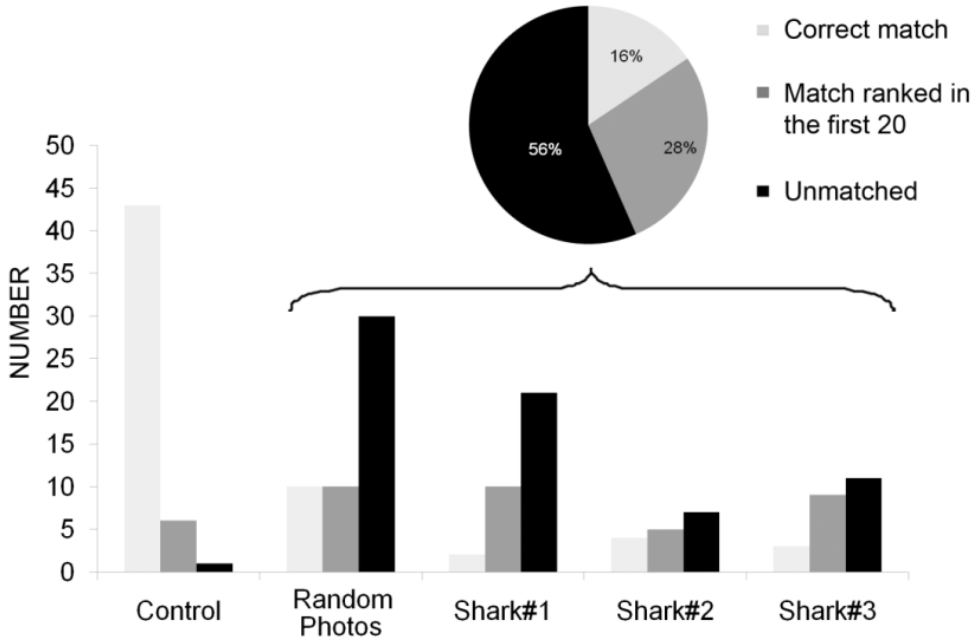

Figure S5 DARWIN results showing the number of matched images for each trial conducted. The pie chart indicates the percentage of each score.

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