

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

## Vocal individuality and species divergence in the contact calls of banded penguins

### This is the author's manuscript

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1560483> since 2022-12-21T11:27:46Z

*Published version:*

DOI:10.1016/j.beproc.2016.04.010

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



# UNIVERSITÀ DEGLI STUDI DI TORINO

***This is an author version of the contribution published on:***

*Questa è la versione dell'autore dell'opera:*

*[Behavioural Processes, 2016, 28 83–88]*

***The definitive version is available at:***

*La versione definitiva è disponibile alla URL:*

*[<http://dx.doi.org/10.1016/j.beproc.2016.04.010>]*

## 1 Highlights

- 2 • Acoustic cues of individuality and species were investigated in penguin contact calls.
- 3 • The source-filter theory is a valuable framework for studying bird calls.
- 4 • Contact calls of banded penguins encode individual identity information.
- 5 • Contact calls of congeneric *Spheniscus* penguins have diverged among species.

**Vocal individuality and species divergence in the contact calls of banded penguins**

Livio Favaro<sup>a, \*</sup>, Claudia Gili<sup>b</sup>, Cristiano Da Rugna<sup>c</sup>, Guido Gnone<sup>b</sup>, Chiara Fissore<sup>a</sup>, Daniel Sanchez<sup>d</sup>, Alan G. McElligott<sup>e</sup>, Marco Gamba<sup>a</sup>, Daniela Pessani<sup>a</sup>

<sup>a</sup> Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy

<sup>b</sup> Acquario di Genova, Costa Edutainment SpA, Genova, Italy

<sup>c</sup> Acquario di Cattolica, Costa Edutainment SpA, Cattolica, Rimini, Italy

<sup>d</sup> Zoom Torino, Cumiana, Turin, Italy

<sup>e</sup> Queen Mary University of London, Biological and Experimental Psychology, School of Biological and Chemical Sciences, London, U.K.

\* Corresponding author

*Email address:* livio.favaro@unito.it (Livio Favaro)

## Abstract

Penguins produce contact calls to maintain social relationships and group cohesion. Such vocalisations have recently been demonstrated to encode individual identity information in the African penguin. Using a source-filter theory approach, we investigated whether acoustic cues of individuality can also be found in other *Spheniscus* penguins and the acoustic features of contact calls have diverged within this genus. We recorded vocalisations from two *ex-situ* colonies of Humboldt penguin and Magellanic penguin (sympatric and potentially interbreeding in the wild) and one *ex-situ* group of African penguins (allopatric although capable of interbreeding with the other two species in captivity). We measured 14 acoustic parameters from each vocalisation. These included temporal (duration), source-related (fundamental frequency,  $f_0$ ), and filter-related (formants) parameters. They were then used to carry out a series of stepwise discriminant function analyses (with cross-validation) and General Linear Model comparisons. We showed that contact calls allow individual discrimination in two additional species of the genus *Spheniscus*. We also found that calls can be classified according to species in a manner far greater than that attributable by chance, even though there is limited genetic distance among African, Humboldt, and Magellanic penguins. Our results provide further evidence that the source-filter theory is a valuable framework for investigating the biologically meaningful information contained in bird vocalisations. Our findings also provide novel insights into penguin vocal communication and suggest that contact calls of the penguin family are affected by selection for individuality.

**Keywords:** bioacoustics; seabirds; source-filter theory; *Spheniscus*

## 36 1. Introduction

37 Penguins are a monophyletic group of pelagic seabirds that forage at sea and breed on land,  
38 where most of the species form dense colonies (Williams, 1995; Schreiber & Burger, 2002). The  
39 penguin genus *Spheniscus* comprises four living species collectively known as "banded penguins".  
40 The genus can be further divided in two sister taxa: one formed by the Humboldt (*Spheniscus*  
41 *humboldti*) and Galápagos (*S. mendiculus*) penguins and the second by the African (*S. demersus*)  
42 and Magellanic (*S. magellanicus*) penguins. The two sister groups diverged in the Pacific and  
43 Atlantic Oceans approximately 4 million years ago (Baker et al., 2006). However, despite their  
44 genetic relationship, Magellanic and Humboldt penguins are partially sympatric and their  
45 geographical distributions overlap along the Pacific Ocean coast of South America. For example,  
46 in the Chilean islands of Puñihuil and Metalqui both species coexist in significant numbers  
47 (Simeone and Schlatter, 1998; Simeone et al., 2009). By contrast, their closest relatives, African  
48 and Galápagos penguins are allopatric and breed on the African continent and Galápagos  
49 archipelago, respectively.

50  
51 Penguins use vocalisations for individual localisation, recognition, and to maintain social  
52 relationships and group cohesion (Jouventin, 1982; Favaro et al., 2014a). Based on the acoustic  
53 properties of their vocalisations and behavioural context of emission, four categories can be  
54 distinguished in the vocal repertoire of adult penguins (Jouventin, 1982): contact calls (uttered to  
55 maintain cohesion with the group or the partner), agonistic calls (made during fights and in  
56 territorial defence), as well as two types of display songs: complex vocalisations made of syllable  
57 sequences that can be uttered by single birds (ecstatic display songs), or by pairs (mutual display  
58 songs).

59  
60 Penguin display songs are adapted for mate and parent-offspring recognition (Lengagne et al.,  
61 1997; Lengagne et al., 2000; Aubin and Jouventin, 2002) and acoustic cues for individuality have  
62 been found in all the penguin species that have been studied (Aubin and Jouventin, 2002; Aubin,  
63 2004; Clark et al., 2006). The mechanisms used to encode the individual identity information in the

64 display songs are exposed to ecological sources of selection and also vary according to breeding  
65 ecology and the colonial lifestyles of the different species (Aubin, 2004). In particular, non-nesting  
66 species (e.g. King Penguin, *Aptenodytes patagonicus*) use the two-voice system as a principal  
67 means of identifying each other (Aubin et al., 2000), while in nesting species (e.g. Adélie penguin,  
68 *Pygoscelis adeliae* or Gentoo penguin, *Pygoscelis papua*), the pitch of the song, frequency and  
69 relative values of harmonics are the main cues for individual recognition (Jouventin and Aubin,  
70 2002). Despite all the information available on display songs, very limited research has been direct  
71 toward studying the other call types, and in particular contact calls.

72  
73 Contact calls have evolved as social signals to maintain cohesion in stable groups (Cheney et al.,  
74 1996; Kondo and Watanabe, 2009; Bergman and Sheehan, 2013), but also to advertise on the  
75 identity of the sender (Sharpe et al., 2013; Favaro et al., 2015), which is particularly important in  
76 fission-fusion societies (Ramos-Fernández, 2005; Terry et al., 2005; Janik et al., 2006). Moreover,  
77 contact calls can encode a great deal of information about the emitter. There is growing evidence  
78 that these vocalisations have the potential to provide information on sex (Guillette et al., 2010), age  
79 (Favaro et al., 2014b), group membership (Boeckle and Bugnyar, 2012), and even emotional state  
80 (Briefer et al., 2015). They can also contain acoustic cues to species (Gamba et al., 2012a;  
81 Cinkova and Policht, 2014) and population (Buhmann-Deever et al., 2007; Husemann et al., 2014).

82  
83 Although the African, Humboldt and Magellanic penguins are closely related, their ecstatic display  
84 songs are clearly recognisable even to human listeners (Jouventin, 1982). Indeed, this vocalisation  
85 has a significant amount of variation among species, and African and Magellanic penguins are  
86 more similar to each other than to Humboldt penguins (Thumser et al., 1996). Moreover, Thumser  
87 and Ficken (1998) showed that contact calls of *Spheniscus* penguins have very similar acoustic  
88 structure among the different species. They are short vocalizations with a clear harmonic structure,  
89 and that intraspecific variation only exists for the dominant frequency. However, these  
90 observations were based on a very limited number of individuals and vocalisations. A recent study  
91 showed that the contact calls of African penguin have the potential to allow individual

92 discrimination (Favaro et al., 2015). In particular, Favaro et al. (2015) used a Discriminant Function  
93 Analysis (DFA) to show that contact calls in this species differ systematically between individuals  
94 (DFA accuracy = 61.1%) and can be classified according to the emitter in a manner far greater  
95 than that attributable to chance. Nevertheless, whether these vocalisations allow individual  
96 discrimination also in other penguin species and contain other biologically meaningful information  
97 still remains to be tested.

98

99 Bird calls are generated by vibrations of membranes in the syrinx (source, determining the  
100 fundamental frequency, " $f_0$ ") and are subsequently filtered by the suprasyringeal vocal tract (filter,  
101 resulting in amplified frequencies called "formants"). The source-filter theory of mammal vocal  
102 production (Fant, 1969; Taylor and Reby, 2010) has recently been shown to be useful in gaining a  
103 far greater understanding of individual identity information contained in seabird vocalisations  
104 (Hardouin et al., 2014; Favaro et al., 2015). Following the source-filter theory approach, we  
105 determined whether the contact calls of Magellanic and Humboldt penguins have the potential to  
106 allow individual discrimination of the callers. Moreover, we tested whether the acoustic features of  
107 contact calls have diverged across this genus. To this end, we compared calls of Magellanic and  
108 Humboldt penguins (that potentially interbreed in the wild), and the African penguin, which is fully  
109 allopatric.

110

## 111 **2. Methods**

### 112 **2.1 Ethics statement**

113 The research conforms to the Ethical Guidelines for the Conduct of Research on Animals by Zoos  
114 and Aquariums (WAZA, 2005) and complies with all applicable Italian laws. Acoustic recordings  
115 were non-invasive and we made every effort to minimize possible disturbance to the penguins.

116

### 117 **2.2 Study sites and penguins**

118 We recorded nine Magellanic penguins (5 males and 4 females) that were all the adult members  
119 of an *ex-situ* colony housed at the Acquario di Genova, Italy. The colony was imported in 2006



120 from the SELWO Marina Delfinarium (Benalmadena, Spain), but was originally from Argentina  
121 (wild individuals stranded due to an oil spill). In Genoa, the colony was maintained in a communal  
122 indoor exhibit (123 m<sup>2</sup> including a pond of 66 m<sup>2</sup>, water depth maximum 3 m) with three concrete  
123 walls and one facing the visitor corridor made up of glass panels, which allows a combined vision  
124 of open air and underwater penguin activity.

125  
126 Vocalisations of Humboldt penguins were collected from six birds (3 males and 3 females)  
127 belonging to a captive colony housed at the Acquario di Cattolica, Italy. The composition of the  
128 whole colony was 4 males and 8 females. Penguins were housed in an indoor communal exhibit  
129 of 75 m<sup>2</sup>, including a pond of 35 m<sup>2</sup> (maximum depth 2 m). The colony was established at the  
130 Acquario di Cattolica from 2007 to 2009 joining adult penguins from the Schönbrunn Zoo (Austria)  
131 and the North of England Zoological Society, Chester (United Kingdom).

132  
133 We recorded 24 adult African penguins (18 males and 6 females) from a large *ex-situ* colony (26  
134 males, 27 females, 4 juveniles, and 3 chicks hatched during the study period), maintained at the  
135 biopark Zoom Torino, Italy. The colony was established in 2009 by combining several adult African  
136 penguins from four different zoological facilities in Europe (Artis Royal Zoo, Amsterdam, NL; Bird  
137 Park Avifauna, Alphen an den Rijn, NL; Wilhelma Zoo, Stuttgart, DE; South Lake Wild Animal Park,  
138 Manchester, UK). The colony was housed in an outdoor exhibit (1,500 m<sup>2</sup>, including a pond of 120  
139 m<sup>2</sup>, water depth maximum 3 m), which reproduces the habitat of “Boulders Beach”, a natural  
140 nesting site in South Africa.

141  
142 All penguins recorded in this study were habituated to human presence and used to being audio-  
143 and video-recorded during their normal daily activity. Additionally, all birds had a microchip  
144 transponder and a flipper band to allow individual identification.

145

## 146 **2.3 Recordings of vocalisations**

Vocalisations of African penguins were collected over 50 non-consecutive days from September 2014 to March 2015. Recordings of Humboldt penguins were collected over 40 non-consecutive days between May and September 2015. Magellanic penguins were recorded over 30 non-consecutive days from February to April 2015. In all facilities, vocalisations were collected using the all occurrence animal sampling method (Altmann, 1974). Vocalisations were recorded at a distance of between 2 and 10 m from the caller with a RØDE NTG2 Super-Cardioid microphone (frequency response 20 Hz to 20 kHz, sensitivity -36dB +/- 2 dB re 1 V/Pa at 1 kHz, max SPL 131dB). In order to reduce recorded noise, the microphone was mounted on a RØDE PG2 Pistol Grip and protected with a windscreen. We also made every effort to orientate the microphone towards the calling bird. The microphone was connected to a TASCAM DR-680 or TASCAM DR-40 digital recorder (44.1 kHz sampling rate) and acoustic data were saved into an internal SD memory card in WAV format (16-bit amplitude resolution). All the files were then transferred to a laptop computer for later acoustic analyses.

160

## 161 **2.4 Acoustic analysis**

We used narrow-band spectrograms to visually inspect the overall spectral structure of audio recordings. In particular, the waveform and the FFT (Fast Fourier Transform) spectrogram were generated with the Praat (Boersma, 2001) sound editor window. We used a customised spectrogram setting (view range = 0 to 8000 Hz, window length = 0.02 s, dynamic range = 50 dB). For the purpose of this study we only selected contact calls (Supplementary Material, Figure S1). However, 355 calls for African penguins, 150 for Humboldt penguins, and 486 for Magellanic penguins were initially discarded because they showed excessive background noise or because calls were overlapping between different penguins vocalising at the same time. Overall, the spectrographic inspection allowed us to select a total of 392 vocalisations for African penguins, 119 for Humboldt penguins, and 976 for Magellanic penguins. The contribution of each penguin to the recordings is presented in the Supplementary Material (Table S1).

173

174 Acoustic measurements on selected vocalisations were carried out using a series of custom scripts

(see Gamba et al., 2012b; Favaro et al., 2015) in Praat v.5.4.08. The abbreviations that we use for the various vocal parameters in this study are based on recent advice regarding this terminology (Titze et al., 2015). For each contact call, we measured a series of spectral and temporal acoustic parameters, which were potentially important to vocal distinctiveness. These included both temporal measures, such as call duration (Dur), source-related vocal features ( $f_0$ ) and filter-related acoustic features (formants; Figure 1). We also quantified the mean harmonics-to-noise ratio value (Sonority). However, before measuring formants, we estimated the approximate vocal tract length (VTL) for banded penguins, to set a plausible number of formants in a given frequency range (Gamba and Giacoma, 2006; Favaro et al., 2015). In particular, we built computational models of the penguin vocal tract deriving information from cadavers of individuals died from natural causes in different zoological facilities in Italy. We modelled vocal tract resonances using a MATLAB-based computer program for vocal tract acoustic response calculation (VTAR, Vocal Tract Acoustic Response; Zhou, 2004). The acoustic response of the vocal tract models and the visual inspection of the spectrograms indicated 5 formants below 3500 Hz for the contact calls.

We extracted the  $f_0$  contour of each call using a cross-correlation method [Sound: To Pitch (cc) command]. Depending on the acoustic characteristics of each vocal type, we used a time step of 0.01 s, a pitch floor of 150 Hz, and a pitch ceiling of 350 Hz. From each extracted  $f_0$  contour, we obtained the frequency value of  $f_0$  at the start ( $f_0$  start) and at the end ( $f_0$  end) of the call; the mean ( $f_0$  mean), minimum ( $f_0$  min) and maximum ( $f_0$  max) fundamental frequency values across the call. We measured the percentage of duration from the beginning of the signal to the time at which the minimum frequency (Time  $f_0$  min) and the maximum frequency (Time  $f_0$  max) occurs. Finally, we extracted the contour of the first four formants ( $F_1$ - $F_4$ ) of each call using a Linear Predictive Coding analysis (LPC; Sound: To Formant (burg) command; time step = 0.045 s, maximum number of formants = 5, maximum formant = 3500 Hz) and we calculated the average frequency values. In addition, we calculated the formant dispersion ( $\Delta F$ ) using the methods described by Reby and McComb (2003).

## 203    **2.5 Statistical Analysis**

204    We performed two separate cross-validated (leave-one-out) discriminant function analyses (DFA)  
205    for Humboldt and Magellanic penguins to investigate whether contact calls could be used to  
206    discriminate among individuals in these two species. In both analyses, the caller was used as the  
207    group identifier and the acoustic variables as discriminant variables. We used a feed forward  
208    procedure with default F-values threshold in SPSS v.20 for acceptance or rejection of the  
209    discriminant variables. Moreover, the coefficients of classification were corrected according to the  
210    group sizes, since the different individuals did not contribute equally to the samples. Finally, for  
211    each vocal parameter, we calculated the Potential of Identity Coding (PIC) using the correction for  
212    small samples (e.g. Charrier et al., 2004). PIC assesses the ratio between within-individual  
213    variation and between-individual variation of an acoustic parameters (Mathevon et al., 2003). If the  
214    ratio is  $> 1$ , then the parameter has the potential to encode the individual identity information, since  
215    its intra-individual variability is smaller than its inter-individual variability. Details for the PIC  
216    calculation are presented in the Supplementary Material (Table S3).

217

218    We performed a cross-validated (leave-one-out) DFA to determine if contact calls of African,  
219    Humboldt, and Magellanic penguins could be correctly classified to the correct species. In this  
220    case, we used the species as test factor and the acoustic parameters as discriminant variables.  
221    Moreover, since we did not record the same number of calls per individual, we also performed a  
222    pDFA (Mundry and Sommer, 2007) for nested data with 1000 permutations. We used the species  
223    as test factor and individuals as a control factor. Finally, since pDFA classification resulted in a  
224    significant discrimination level ( $p < 0.001$ ), we further investigated which vocal parameters  
225    significantly differed in their average values among species using a series of univariate General  
226    Linear Models (GLM) for ANOVA analysis. Specifically, each vocal parameter was entered in turn  
227    as a dependent variable and the species was used as a fixed factor. Individuals were fitted as  
228    random factors to control for repeated sampling. Finally, in order to control for individuality, we  
229    used a nested design with individual factor nested under the dependent variable (species).

230

231 We performed pDFA analysis in R (R Core Team 2014, Version 3.2.2015-04-16, R Foundation for  
232 Statistical Computing). All the other statistical analyses were performed in SPSS v.20 (IBM Corp.  
233 Released 2013. IBM SPSS Statistics for Macintosh, Version 20.0. Armonk, NY: IBM Corp.).

## 234 **3. Results**

### 235 **3.1 Individual identity**

236 Using the source and filter vocal parameters as independent variables, the discriminant function  
237 analysis (DFA) correctly classified 70.0% of the contact calls to the emitter for Magellanic penguins  
238 and 60.5% for Humboldt penguins. The accuracy of the DFA decreased to 69.0% and 55.5%  
239 respectively, when the more conservative leave-one-out cross-validation was applied. The  
240 statistical significance of this classification and details of the canonical discriminant functions are  
241 presented in Supplementary Material (Table S2). Overall, our results show that individual identity is  
242 encoded also in contact calls of both Magellanic and Humboldt penguins. The PIC analysis  
243 confirmed that both source- and filter-related components have the potential to encode individual  
244 identity information (Supplementary Material, Table S3).

### 246 **3.2 Species recognition**

247 Calls were correctly classified to the correct species in 88.8% of cases. The accuracy of the DFA  
248 decreased to 88.3% when the more conservative leave-one-out cross-validation was applied.  
249 Table 1 shows values of the vocal parameters for contact calls of African, Magellanic and  
250 Humboldt penguins. Details of the canonical discriminant functions generated for the discriminant  
251 function analysis are presented in Supplementary Material (Table S4). Figure 2 shows that contact  
252 calls of the three species form distinctive clusters in space, defined by discriminant functions 1 and  
253 2. The nested pDFA confirmed that when controlling for individuality, contact calls could be  
254 correctly classified on the basis of species (cross-validated classification after 1000 permutations:  
255 expected = 41.38%, observed = 77.09%,  $p < 0.001$ ). Results from GLMs (nested ANOVA, Table 2)  
256 showed that average values of eight acoustic parameters significantly differed among species, and  
257 thus are potentially important for vocal distinctiveness. These included call duration, four source-  
258 ( $f_0$  min,  $f_0$  max,  $f_0$  end, Time  $f_0$  min) and three filter- ( $F_1$ ,  $F_4$ ,  $\Delta F$ ) related measures. Therefore, we  
259 found evidence that penguin contact calls also encode species-specific acoustic signatures.

## 260 4. Discussion

261 We investigated whether banded penguin contact calls encode individual identity information and  
262 species-specific vocal signature. We found evidence that contact calls of *Spheniscus* penguins  
263 allow individual discrimination using both source- and filter-related components. Moreover, we  
264 showed that contact calls can be classified according to species in a manner far greater than that  
265 attributable by chance. Overall, our results provide further evidence that the “source-filter” theory of  
266 vocal production (Fitch, 2010; Taylor and Reby, 2010) can be successfully applied to the  
267 interpretation of information contained in bird vocalisations (Ohms et al., 2010; Budka and Osiejuk,  
268 2013).

269  
270 Penguin contact calls have been poorly studied compared to display songs. However, recent  
271 research has found that the contact calls of African penguins are individually distinctive (Favaro et  
272 al., 2015). In this species, the morphology and size of the vocal apparatus allow modifications of  
273 the energy distribution across the spectrum. Accordingly, several source- and filter-related  
274 vocalization features were found to exhibit a smaller amount of intra-individual variation when  
275 compared to inter-individual variation (Favaro et al., 2015). Our results provide further evidence  
276 that individual identity information is also encoded in contact calls of two other species of the  
277 genus *Spheniscus*. The results of the PIC and DFA analyses also support the hypothesis that  
278 vocal individuality in nesting penguins is determined by both source- and filter- related parameters.

279  
280 Banded penguins often form flocks at sea for travelling and foraging (Wilson and Wilson, 1990).  
281 When in flocks, they utter contact calls to maintain group cohesion (Jouventin, 1982). Penguins at  
282 sea have highly synchronised diving behaviour (Siegfried et al., 1975), both when swimming to  
283 travel (short and shallow dives of 10-20 seconds) and when diving for foraging (longest dives up to  
284 2-3 minutes), (Wilson and Wilson, 1990). When underwater, birds are likely to get out of contact  
285 with the other group members and, especially when in large flocks, they can surface  
286 asynchronously (Wilson et al., 1986). In such circumstances, they need to call to locate other  
287 group members when out of sight. In this fission-fusion context, where the effective distance of

288 visual signals is shorter compared to vocalisations, we expected to find high selective pressures  
289 for vocal individuality (Janik and Slater, 1998). In more confined captive settings, juveniles banded  
290 penguins swimming alone in ponds emit contact calls to maintain social contact with parents  
291 (Thumser and Ficken, 1998). In all the colonies studied, we also observed adult pair members  
292 keeping in touch with each other with contact calls when visually isolated and returning to the nest  
293 after that the partner had repeatedly emitted contact calls (L. Favaro, personal observation).  
294 Overall, our results support the hypothesis that penguin contact calls are social signals that have  
295 evolved to facilitate social reunion and maintain group cohesion.

296

297 The ecstatic display songs of African, Magellanic and Humboldt penguins have diverged in several  
298 spectral and temporal acoustic parameters (Thumser and Ficken, 1998) and it has been suggested  
299 that this vocal type can reflect phylogenetic relationships within this genus (Thumser et al., 1996).  
300 Our DFA analysis results support the hypothesis, demonstrating that acoustic cues to species are  
301 also present in contact calls and are likely to depend on the anatomy of the vocal tract. In addition,  
302 the GLM underlined how eight source- and filter- vocal parameters differ among the three species.  
303 However, in closely related seabirds, the ability to detect species-specific vocal features is  
304 controversial (Bretagnolle and Robisson, 1991; Cureé et al., 2010; Curé et al., 2012) and there is  
305 no evidence that penguins are capable of using such information from vocalisations. In particular,  
306 penguins of the genus *Spheniscus* have limited phylogenetic distance and hybrids have been  
307 found where the geographical distributions of Magellanic and Humboldt penguins overlap along the  
308 Pacific coast of South America (Simeone and Schlatter, 1998; Simeone et al., 2009). This  
309 suggests that the species-specific recognition systems fail to prevent heterospecific confusion.  
310 Banded penguins are also known to readily hybridise when grouped together in captivity  
311 (McCarthy, 2006). In sympatric closely-related species, differences in signalling characters often  
312 evolve to prevent incorrect mate choice (Losos and Leal, 2013). However, although we found  
313 acoustic differences in both sympatric and allopatric penguins within the penguin genus  
314 *Spheniscus*, we suggest that species-specific acoustic signature in their contact calls is more likely  
315 to be a by-product of divergent ecological selection rather than a pre-zygotic mechanism to prevent



316 hybridization. In social animals, not all the acoustic cues encoded in calls are necessarily salient to  
317 receivers (Townsend et al., 2011). We recommend additional studies using playback experiments,  
318 to further investigate the sensitivity of penguins to acoustic cues of species.

319

320 In conclusion, our findings confirm that the source-filter theory of vocal production can be  
321 successfully adopted to study bird vocalisations. Using this approach, we provide further evidence  
322 that banded penguin contact calls encode individual identity and species-specific signatures. We  
323 suggest that the high levels of individuality in these vocalisations are an adaptation to travelling  
324 and foraging in large flocks at sea.

325

## 326 **Acknowledgements**

327 Special thanks are due to Chiara Alfieri, Gilda Todino and all the students who helped with audio  
328 recordings. We are grateful to Sergio Castellano for statistical advice. We thank the penguin  
329 caregivers of the Acquario di Cattolica, Acquario di Genova, and Zoom Torino. We are grateful to  
330 two anonymous reviewers for constructive observations, which helped improve the manuscript.  
331 Livio Favaro was financially supported by the University of Torino through a MIUR co-financed  
332 postdoctoral fellowship.

333

## 334 **References**

- 335 Altmann J. Observational study of behavior: sampling methods. *Behaviour*. 1974;49: 227-267.
- 336 Aubin T. Penguins and their noisy world. *An Acad Bras Cienc*. 2004;76: 279–283.
- 337 Aubin T, Jouventin P. How to vocally identify a kin in a crowd: the penguin model. *Adv Stud Behav*.  
338 2002;31: 243-277.
- 339 Aubin T, Jouventin P, Hildebrand C. Penguins use the two-voice system to recognize each other.  
340 *Proc R Soc B*. 2000;267: 1081–1087.
- 341 Baker AJ, Pereira SL, Haddrath OP, Edge KA. Multiple gene evidence for expansion of extant  
342 penguins out of Antarctica due to global cooling. *Proc R Soc B*. 2006;273: 11-17.

343 Bergman TJ, Sheehan MJ. Social knowledge and signals in primates. *Am J Primatol.* 2013;75:  
344 683-694.

345 Boeckle M, Bugnyar T. Long-term memory for affiliates in ravens. *Curr. Biol.* 2012;22: 801-806.

346 Boersma P. Praat, a system for doing phonetics by computer. *Glott International.* 2001;5:9/10: 341-  
347 345.

348 Bretagnolle V, Robisson P. Species-specific recognition in birds - an experimental investigation of  
349 Wilsons storm-petrel (Procellariiformes, Hydrobatidae) by means of digitalized signals. *Can J*  
350 *Zool* 1991;69: 1669-1673.

351 Briefer EF, Tettamanti F, McElligott AG. Emotions in goats: mapping physiological, behavioural  
352 and vocal profiles. *Anim Behav.* 2015;99: 131-143.

353 Budka M, Osiejuk TS. Formant frequencies are acoustic cues to caller discrimination and are a  
354 weak indicator of the body size of corncrake males. *Ethology.* 2013; 119: 960–969.

355 Buhrmann-Deever SC, Rappaport AR, Bradbury JW. Geographic variation in contact calls of feral  
356 North American populations of the Monk Parakeet. *Condor.* 2007;109, 389-398.

357 Charrier I, Bloomfield LL, Sturdy CB. Note types and coding in parid vocalizations. I: The chick-a-  
358 dee call of the black-capped chickadee (*Poecile atricapillus*). *Can J Zool.* 2004;82: 769–779.

359 Cheney DL, Seyfarth RM, Palombit R. The function and mechanisms underlying baboon ‘contact’  
360 barks. *Anim Behav.* 1996;52: 507-518.

361 Cinkova I, Policht R. Contact calls of the northern and southern white rhinoceros allow for  
362 individual and species identification. *PLoS ONE.* 2014;9: e98475.

363 Clark JA, Boersma PD, Olmsted DM. Name that tune: call discrimination and individual recognition  
364 in Magellanic penguins. *Anim Behav.* 2006;72: 1141–1148.

365 Curé C, Aubin T, Mathevon N. Intra-sex vocal interactions in two hybridizing seabird species  
366 (*Puffinus* sp.). *Behav Ecol Sociobiol.* 2010;64: 1823-1837.

367 Curé C, Mathevon N, Mundry R, Aubin T. Acoustic cues used for species recognition can differ  
368 between sexes and sibling species: evidence in shearwaters. *Anim Behav.* 2012;84: 239–250.

369 Fant G. Acoustic theory of speech production. The Hague: Mouton; 1969.

370 Favaro L, Ozella L, Pessani D. The vocal repertoire of the African Penguin (*Spheniscus*  
371 *demersus*): structure and function of calls. PLoS ONE. 2014a;9(7): e103460. doi:  
372 10.1371/journal.pone.0103460

373 Favaro L, Briefer EF, McElligott AG. Artificial Neural Network approach for revealing individuality,  
374 group membership and age information in goat kid contact calls. Acta Acust united Ac.  
375 2014b;100: 782–789. doi: 10.3813/AAA.918758

376 Favaro L, Gamba M, Alfieri C, Pessani D, McElligott AG. Vocal individuality cues in the African  
377 penguin (*Spheniscus demersus*): a source-filter theory approach. Sci Rep. 2015;5: 17255. doi:  
378 10.1038/srep17255

379 Fitch WT. The evolution of language. Cambridge: Cambridge University Press; 2010.

380 Gamba M, Giacoma C. Vocal tract modeling in a prosimian primate: the black and white ruffed  
381 lemur. Acta Acust united Ac. 2006;92: 749-755.

382 Gamba M, Colombo C, Giacoma C. Acoustic cues to caller identity in lemurs: a case study. J Ethol.  
383 2012a;30: 191-196.

384 Gamba M, Friard O, Giacoma C. Vocal tract morphology determines species-specific features in  
385 lemur vocal signals (*Eulemur*). Int J Primatol. 2012b;33: 1453-1466.

386 Guillette LM, Bloomfield LL, Batty ER, Dawson MRW, Sturdy CB. Black-capped (*Poecile*  
387 *atricapillus*) and mountain chickadee (*Poecile gambeli*) contact call contains species, sex, and  
388 individual identity features. J Acoust Soc Am. 2010;127: 1116 -1123.

389 Hardouin LA, Thompson R, Stenning M, Reby D. Anatomical bases of sex- and size-related  
390 acoustic variation in herring gull alarm calls. J Avian Biol. 2014;45: 157-166.

391 Husemann M, Ulrich W, Habel JC. The evolution of contact calls in isolated and overlapping  
392 populations of two white-eye congeners in East Africa (Aves, *Zosterops*). BMC Evol Biol.  
393 2014;14: 115.

394 Janik VM, Slater PJB. Context-specific use suggests that bottlenose dolphin signature whistles are  
395 cohesion calls. Animl Behav. 1998; 56: 829–838.

396 Janik VM, Sayigh LS, Wells RS. Signature whistle shape conveys identity information to bottlenose  
397 dolphins. Proc Natl Acad Sci USA. 2006;103: 8293-8297.

398 Jouventin P. Visual and vocal signals in penguins, their evolution and adaptive characters. *Adv*  
399 *Ethol.* 1982;58: 3–148.

400 Jouventin P, Aubin T. Acoustic systems are adapted to breeding ecologies: individual recognition  
401 in nesting penguins. *Anim Behav.* 2002;64: 747–757.

402 Kondo N, Watanabe S. Contact calls: information and social function. *Jpn Psychol Res* 2009;51:  
403 197–208.

404 Lengagne T, Lauga J, Jouventin P. A method of independent time and frequency decomposition of  
405 bioacoustic signals: inter-individual recognition in four species of penguins. *CR Acad Sci Ser III*  
406 *Sci Vie.* 1997;320: 885-891.

407 Lengagne T, Aubin T, Jouventin P, Lauga J. Perceptual salience of individually distinctive features  
408 in the calls of adult king penguins. *J Acoust Soc Am.* 2000;107: 508-516.

409 Losos JB, Leal M. The evolution of species recognition signals. *Mol Ecol.* 2013;22: 3879–3881.

410 Mathevon N, Charrier I, Jouventin P. Potential for individual recognition in acoustic signals: a  
411 comparative study of two gulls with different nesting patterns. *C R Biol.* 2003; 326: 329-337.

412 McCarthy EM. *Handbook of Avian Hybrids of the World.* New York: Oxford University Press; 2006.

413 Mundry R, Sommer C. Discriminant function analysis with nonindependent data: Consequences  
414 and an alternative. *Anim Behav.* 2007;74: 965–976.

415 Ohms VR, Snelderwaard PC, ten Cate C, Beckers GJL. Vocal tract articulation in Zebra Finches.  
416 *PLoS ONE.* 2010;5(7): e11923.

417 Ramos-Fernández G. Vocal communication in a fission-fusion society: do spider monkeys stay in  
418 touch with close associates? *Int J Primatol.* 2005;26: 1077-1092.

419 Reby D, McComb K. Anatomical constraints generate honesty: acoustic cues to age and weight in  
420 the roars of red deer stags. *Anim Behav.* 2003;65: 519-530.

421 Schreiber EA, Burger J. *Biology of Marine Birds.* Boca Raton: CRC Press; 2002.

422 Sharpe LL, Hill A, Cherry MI. Individual recognition in a wild cooperative mammal using contact  
423 calls. *Anim Behav.* 2013;86: 893–900.

424 Siegfried W, Frost P, Kinahan J, Cooper J. Social behaviour of Jackass Penguins at sea. *Zoo Afr.*  
425 1975;10: 87–100.

426 Simeone A, Schlatter R. Threats to mixed-species colony of *Spheniscus* penguins in southern  
 427 Chile. *Colon Waterbirds*. 1998;21: 418-421.

428 Simeone A, Hiriart-Betrand L, Reyes-Arriagada R, Halpern M, Dubach J, Wallace R, et al.  
 429 Heterospecific pairing and hybridization between wild Humboldt and Magellanic Penguins in  
 430 southern Chile. *Condor*. 2009;111: 544-550.

431 Taylor AM, Reby D. The contribution of source-filter theory to mammal vocal communication  
 432 research. *J Zool*. 2010;280: 221-236.

433 Terry AMR, Peake TM, McGregor PK. The role of vocal individuality in conservation. *Front Zool*.  
 434 2005;2: 10.

435 Thumser NN, Ficken MS. A comparison of the vocal repertoires of captive *Spheniscus* penguins.  
 436 *Mar Ornithol*. 1998;26: 41–48.

437 Thumser NN, Karron JD, Ficken MS. Interspecific variation in the call of *Spheniscus* penguins.  
 438 *Wilson Bull*. 1996;108: 72-79.

439 Titze IR, Baken RJ, Bozeman KW, Granqvist S, Henrich N, Herbst CT, et al. Toward a consensus  
 440 on symbolic notation of harmonics, resonances, and formants in vocalization. *J Acoust Soc*  
 441 *America*. 2015;137: 3005-3007.

442 Townsend S, Hollen L, Manser M. Meerkat close calls encode group-specific signatures, but  
 443 receivers fail to discriminate. *Anim Behav*. 2011;80: 133-138.

444 WAZA. Ethical guidelines for the conduct of research on animals by zoos and aquariums. 60<sup>th</sup>  
 445 Annual Conference of the World Association of Zoos and Aquariums, New York (USA), 2005.  
 446 Available: <http://www.waza.org/en/site/conservation/code-of-ethics-and-animal-welfare>.

447 Williams TD. *The Penguins*. Oxford: Oxford University Press; 1995.

448 Wilson RP, Wilson MPT. Foraging ecology of breeding *Spheniscus* penguins. In: Davis L, Darby  
 449 JT, editors. *Penguin biology*. London: Academic Press. 1990; 181–206.

450 Wilson RP, Wilson MPT, McQuaid L. Group size in foraging African penguins (*Spheniscus*  
 451 *demersus*). *Ethology*. 1986;72: 338–341. doi:10.1111/j.1439-0310.1986.tb00634.x.

452 Zhou X, Zhang Z, Espy-Wilson CY. VTAR: A Matlab-based computer program for vocal tract  
 453 acoustic modeling. *J Acoust. Soc. Am*. 2004;115: 2543.

454 **Table 1.** Values of the vocal parameters (mean  $\pm$  SD) for the contact calls of each penguin species.

<b>Vocal parameter</b>	<b>African<sup>*</sup> (n = 392)</b>	<b>Humboldt<sup>**</sup> (n = 119)</b>	<b>Magellanic<sup>***</sup> (n = 976)</b>
Dur (s)	0.57 $\pm$ 0.12	0.91 $\pm$ 0.20	0.86 $\pm$ 0.16
$f_0$ mean (Hz)	275 $\pm$ 22	253 $\pm$ 16	270 $\pm$ 23
$f_0$ max (Hz)	299 $\pm$ 21	302 $\pm$ 22	291 $\pm$ 21
$f_0$ min (Hz)	248 $\pm$ 25	222 $\pm$ 20	246 $\pm$ 23
$f_0$ start (Hz)	267 $\pm$ 29	275 $\pm$ 29	267 $\pm$ 25
$f_0$ end (Hz)	279 $\pm$ 28	278 $\pm$ 27	267 $\pm$ 25
Time $f_0$ min (%)	69 $\pm$ 34	50 $\pm$ 26	51 $\pm$ 40
Time $f_0$ max (%)	41 $\pm$ 34	49 $\pm$ 43	39 $\pm$ 35
Sonority	8.63 $\pm$ 5.98	4.9 $\pm$ 3.54	7.43 $\pm$ 3.02
F <sub>1</sub> (Hz)	572 $\pm$ 133	450 $\pm$ 109	599 $\pm$ 80
F <sub>2</sub> (Hz)	1030 $\pm$ 143	976 $\pm$ 96	978 $\pm$ 88
F <sub>3</sub> (Hz)	1668 $\pm$ 200	1660 $\pm$ 123	1625 $\pm$ 118
F <sub>4</sub> (Hz)	2468 $\pm$ 230	2475 $\pm$ 148	2431 $\pm$ 98
$\Delta F$	697 $\pm$ 65	691 $\pm$ 45	683 $\pm$ 31

455 \* 24 individuals; \*\* 6 individuals; \*\*\* 9 individuals

456 **Table 2.** GLM results (ANOVA analysis) for the effects of penguin species on the contact call  
 457 acoustic variables.

Vocal parameter	df	F	Significance
Dur	2	30.08	p < 0.001
$f_0$ mean	2	2.73	ns
$f_0$ max	2	3.90	p < 0.05
$f_0$ min	2	4.04	p < 0.05
$f_0$ start	2	1.28	ns
$f_0$ end	2	5.46	p < 0.05
Time $f_0$ min	2	4.27	p < 0.05
Time $f_0$ max	2	1.18	ns
Sonority	2	1.75	ns
F <sub>1</sub>	2	14.07	p < 0.001
F <sub>2</sub>	2	2.46	ns
F <sub>3</sub>	2	2.52	ns
F <sub>4</sub>	2	3.51	p < 0.05
$\Delta F$	2	3.27	p < 0.05

458 Identity was nested into species and included as a random factor. Species was included as a fixed  
 459 factor.

460 **Figure captions**

461

462 **Figure 1.** Spectrogram (Gaussian window shape, view range = 0 to 5000 Hz, window length =  
463 0.05 s, dynamic range = 70 dB, time step = 0.004 s, frequency step = 20 Hz) and LPC spectrum  
464 (500 Hz cepstral smoothed) of contact call (African penguin) showing  $f_0$  and formants ( $F_1$ - $F_4$ ).

465

466 **Figure 2.** Discriminant scores generated by the discriminant functions 1 and 2 to classify contact  
467 calls of African (*S. demersus*), Humboldt (*S. humboldti*), and Magellanic (*S. magellanicus*)  
468 penguins. Black dots indicate the centroid of each species.



Figure 1  
[Click here to download high resolution image](#)

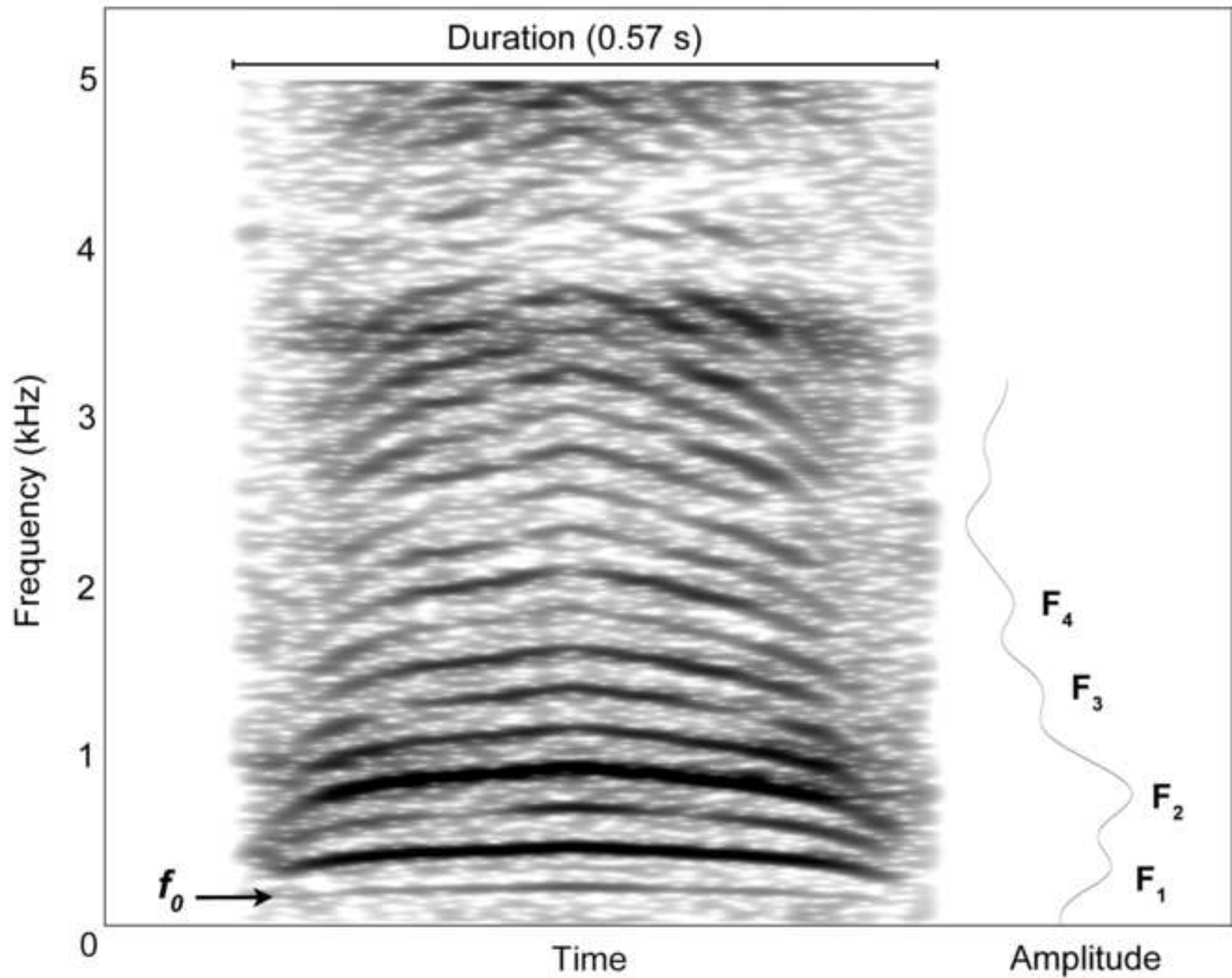
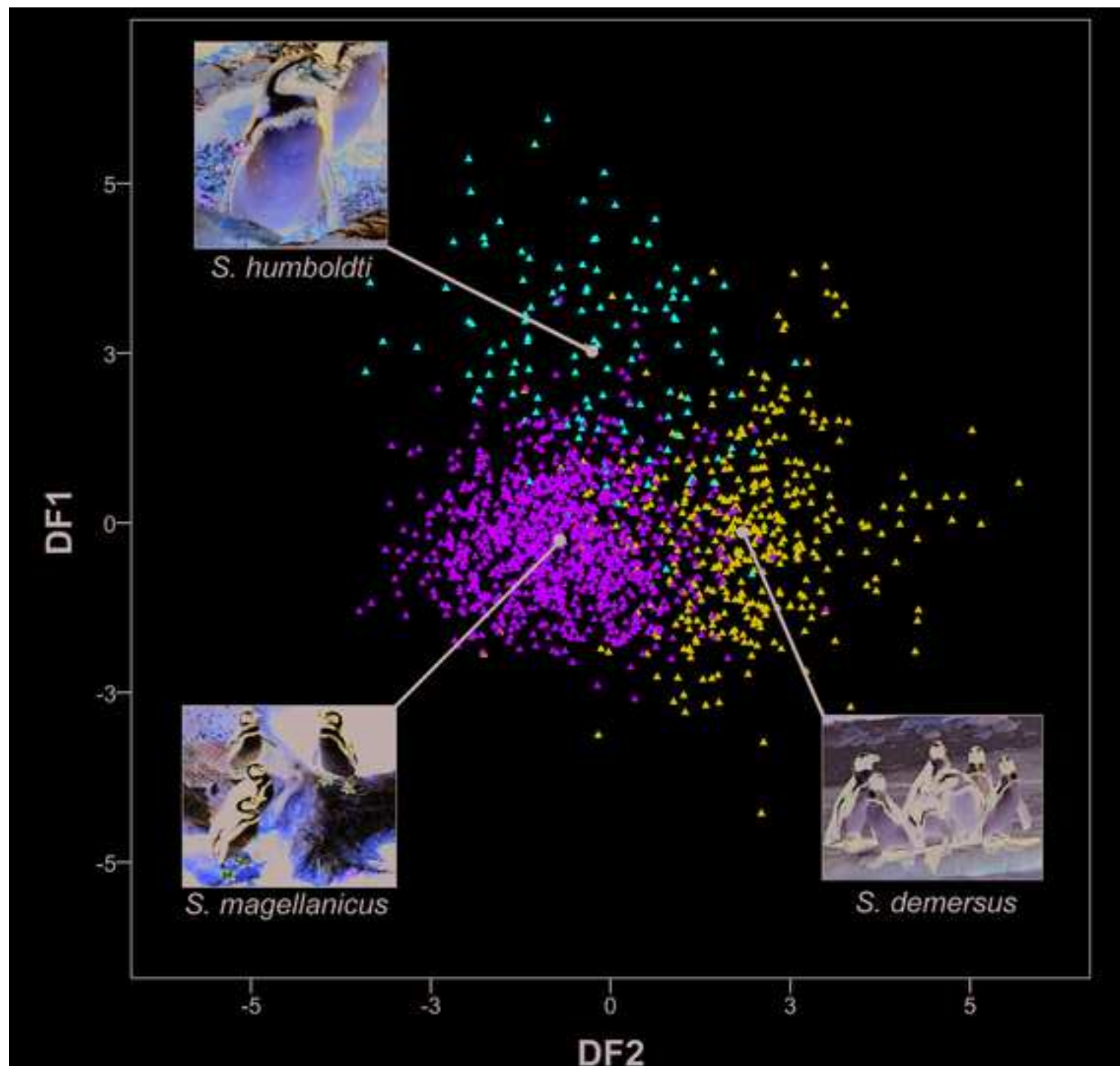


Figure 2  
[Click here to download high resolution image](#)



Supplementary material for on-line publication only

[Click here to download Supplementary material for on-line publication only: SEM\\_DEF.pdf](#)