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1   Behavioral evolution in penguins does not reflect phylogeny

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27

28   **Abstract**

29   Over the last two decades, behavioral biologists and ecologists have made effective

30   use of the comparative method but have often stopped short of adopting an explicitly

31   phylogenetic approach. We examined 68 behavior and life history (BLH) traits of 15 penguin

32   species to: 1) infer penguin phylogeny, 2) assess homology of behavioral characters, and 3)

33   evaluate hypotheses about character evolution and ancestral states. Parsimony analysis of the

34   BLH dataset found either two shortest trees (characters coded as unordered) or a single

35   shortest tree (characters coded as a combination of unordered and dollo). The BLH data had

36   significant structure. Kishino-Hasegawa tests indicated that BLH trees were significantly

37   different from most previous estimates of penguin phylogeny. The BLH phylogeny generated

38   from dollo characters appeared to be less accurate than the tree derived from the completely

39   unordered dataset. Dividing BLH data into display and non-display traits resulted in no

40   significant differences in level of homoplasy and no difference in the accuracy of phylogeny.

41   Tests for homology of BLH traits were performed by mapping the characters onto a molecular

42   tree. Assuming that independent gains are less likely than losses of character states, 65 of the

43   68 characters were likely to be homologous across taxa and at least several characters

44   appeared to have been stable since the origin of modern penguins around 30 million years.

45   Finally, the likely BLH traits of the most recent common ancestor of extant penguins were

46   reconstructed from character states along the internal branch leading to the penguins. This

47   analysis suggested that the 'proto-penguin' probably had a similar life history to current

48   temperate penguins but few ritualised behaviors. A southern, cool- temperate origin of

49   penguins is suggested.

50

51

52

53   Despite a "flowering in phylogenetics" (Doyle, 1993), most comparative behavioral

54   studies have taken the 'convergence approach' (Winkler, 2000) where correlations among

55   similar events across lineages are explored using one of several methods (e.g., Gittleman and

56   Kot, 1990; Harvey and Pagel, 1991). Fewer behavioral studies have taken the 'homology

57   approach' (Winkler, 2000) where unique events within lineages are explored and similar traits

58   in different species are assumed to be homologous. On this basis Lorenz (1958) and

59   Tinbergen (1959) claimed that behavioral characters could be used to infer evolutionary

60   relationships (see also Hinde and Tinbergen, 1965; Brooks and McLennan, 1991). Critics of

61   the use of behavioral characters, however, pointed out that similarities among behavioral traits

62   were not always indicative of homology. They claimed that behavior may be more prone to

63   convergence than other types of traits (Atz, 1970; Aronson, 1981). As Tinbergen (1959)

64   himself stated,

65   "I suggest that it is *because of the limited number of possibilities* of doing 'the

66   opposite' to showing preparedness to attack, that we find some curious similarities

67   in the defensive threat and appeasement postures of widely separated species."

68   [author's italics].

69   Other often-cited problems with investigations of behavioral evolution are the

70   supposed instability, evanescence, lack of character independence, and small likelihood of

71   fossilisation of behavioral characters (Atz, 1970). Thus, a major challenge for those who

72   championed use of behavioral characters was to demonstrate the homology of behavioral

73   characters. Only characters that have arisen once can be considered to be homologous

74   (Wagner, 1989), that is, traits are shared due to common ancestry (see Nixon and Carpenter,

75   2012). As suggested by Japyassu and Machado (2010), and following Brower and

76   Schawaroch (1996), assigning behavioural homology is a three--stage process where

77   comparable features are discovered among taxa, then character variability is partitioned into

78   subunits. These two steps identify phylogenetic homology (Nixon and Carpenter, 2012).

79 Finally, congruence between character state distribution and a phylogenetic hypothesis, such

80 as a cladogram, is obtained either confirming the homology or identifying homoplasy (either

81 through convergence or observational error (Nixon and Carpenter, 2012)). Using parsimony-

82 based phylogenetic methods, character homology can be assessed by mapping a character onto

83 an independent tree to assess whether it has arisen more than once (Brooks and McLennan,

84 1991). The phylogenetic content of a behavioral dataset can also be evaluated by comparing

85 trees based on behavior with independently derived trees.

86   In a renaissance of phylogenetic behavioral studies lead by Brooks and McLennan

87 (1991), many studies concentrated on testing hypotheses of behavioral homology by mapping

88 behaviors onto a genetic or morphological tree (e.g., Beehler and Swaby, 1991; Langtimm and

89 Dewsbury, 1991; Packer, 1991; McKitrick, 1992; Sillen-Tullberg and Møller, 1993; Winkler

90 and Sheldon, 1993; Price and Lanyon, 2002). Other studies used behavioral characters, or a

91 mixture of behavioral and other characters, to reconstruct the phylogenetic relationships of

92 organisms (e.g., Cracraft, 1985; McLennan et al., 1988; Arntzen and Sparreboom, 1989;

93 Prum, 1990; Crowe et al., 1992; Proctor, 1992; Paterson et al., 1995; Kennedy et al., 1996;

94 Slikas, 1998; Stuart and Hunter, 1998; McLennan and Mattern, 2001; Stuart and Currie, 2001;

95 Noll, 2002; Mattern and McLennan, 2004; Robillard et al., 2006). These studies have

96 generally shown relatively low levels of convergence (e.g., Prum, 1990; de Queiroz and

97 Wimberger, 1993; Paterson et al., 1995; Kennedy et al., 1996; Slikas, 1998; McLennan and

98 Mattern, 2001; Stuart and Currie, 2001), and high levels of congruence between trees derived

99 from behavior and different character types (e.g., McLennan et al., 1988; Arntzen and

100 Sparreboom, 1989; Prum, 1990; Paterson et al., 1995; Kennedy et al., 1996; Slikas, 1998;

101 Noll, 2002; Stuart et al., 2002). Highly predictable, and often stereotypical, behaviors and life

102 history traits present in all appropriate individuals are assumed to be inherited and, if found in

103 different species, are potential homologues (Hapyassu and Machado, 2010). De Queiroz and

104 Wimberger (1993) examined multiple datasets and demonstrated that there was no more

105   homoplasy in behavioral traits than morphological traits. Similarly, in a study examining

106   multiple datasets for seabird species, Paterson et al. (1995) showed that retention indices of

107   the molecular datasets were not significantly higher than that of behavioral data. Several

108   techniques may assess whether congruence between behavioral and other trees is significant,

109   from the strictly topological based (e.g., Page, 1990; Page, 1992) to those that examine the

110   underlying uncertainty of the data generating the tree (e.g., Kishino and Hasegawa, 1989).

111   Here, we use behavior and life history (BLH) characters to derive a phylogeny for

112   penguins (Order Sphenisciformes) and to assess the accuracy of this phylogeny by comparing

113   with those constructed from other data types. Penguins are a homogenous group of seabirds

114   that became extremely modified for swimming with the complete loss of aerial flight (Bertelli

115   and Giannini, 2005). There has never been any doubt over monophyly of spenisciforms,

116   which are very distinct from all possible sister groups with an origin in excess of 60 million

117   years ago (Clarke et al, 2007).

118   Congruence among trees generated from independent datasets will generally indicate

119   common phylogenetic history unless there has been lineage sorting, hybridisation of taxa

120   and/or non-independence of characters (Penny et al., 1982; Bledsoe and Raikow, 1990; Zink

121   and Avise, 1990; Paterson et al., 1993; Miyamoto and Fitch, 1995). The degree of congruence

122   among phylogenies generated from penguin BLH data and other character types will indicate

123   how useful BLH characters are in reconstructing phylogeny. Each BLH character is examined

124   for homology by calculating the number of times it is gained over a tree based on molecular

125   data from several gene regions. Finally, evolution of BLH traits is examined by the

126   reconstruction of ancestral BLH character states.

127

128   *Penguin phylogeny*

129   To assess the accuracy of the phylogeny derived from BLH characters, phylogenies

130   derived from other character types are required. The relationships of penguins have long been

131   contentious. While it is generally accepted that the order is monophyletic and contains six

132   extant genera, only a few studies have examined the relationships among these genera. The

133   studies that have investigated the relationships of most extant penguin species include: a

134   phenetic study of myology (Schreiweis, 1972), a study of behavior with no formal character

135   analysis (Jouventin, 1982), a study of integumentary and breeding characters (Giannini and

136   Bertelli, 2004), phenetic studies of both external morphology and skeletal measurements

137   (Livezey, 1989), a molecular phylogenetic study of several gene regions (Baker et al., 2006),

138   cladistic studies of general morphology and two gene regions (Bertelli and Giannini, 2005)

139   and a parsimony analysis of skeletal traits (Ksepka and Clarke, 2010). Generic level

140   relationships have been investigated using a cladistic study of myology (McKitrick, 1991), a

141   cladistic study of osteology (O'Hara, 1989), a phenetic analysis of DNA-DNA hybridization

142   (Sibley and Ahlquist, 1990) and a likelihood analysis of gene regions (Baker et al., (2006).

143   Each of these analyses produces a different phylogeny for the penguin genera and species

144   (Fig. 1). This lack of congruence is perhaps to be expected, as penguins are highly adapted to

145   the marine environment and under intense convergent selection pressure in their morphology.

146   In studies of morphology and behaviour, phylogenetic signal may thus be obscured by

147   adaptation. Molecular traits, such as the gene regions examined by Baker et al. (2006) and

148   BLH (particularly terrestrial) traits, may be more appropriate for reconstructing penguin

149   phylogeny.

150

151   *Homology testing*

152   In addition to assessing homology of individual BLH characters it is useful to know

153   whether certain types of BLH characters are, on average, more likely to show homoplasy than

154   others. End-products of behavior may show more homoplasy (e.g. caddisfly cases; (Stuart and

155   Currie, 2002), or be as reliable as other character types (e.g. ovenbird nests; (Zyskowski and

156   Prum, 1999). Lorenz (1935; 1941) suggested that behavioral displays are often more useful as

157   phylogenetic characters than other BLH traits, although Tinbergen (1959) argued that, on

158   average, displays would contain no more phylogenetic information than other BLH traits.

159   Paterson et al. (1995) found that seabird agonistic and reproductive displays contained no

160   more phylogenetic information than foraging behavior and life history characters. This lack of

161   difference between display and non-display BLH traits in seabirds may also be the same for

162   penguins. There have, however, been more detailed studies of penguin displays than of

163   seabird displays (e.g., Jouventin, 1982), and this may influence the phylogenetic content of

164   such information. Ksepka and Clarke (2010) also used five behaviour and life history traits in

165   their large morphological analysis of penguins.

166

167   *Character evolution*

168   Characters states do not arise *de novo* but are assembled over evolutionary time in a

169   particular sequence (McLennan, 1991). This is especially apparent in the evolution of

170   behavior (Brooks and McLennan, 1991; McLennan, 1991; McKitrick, 1992). Most

171   phylogenetic work on behavior has involved reconstructing evolutionary sequences and co-

172   occurring displays. These studies include: courtship sequence of newts (Arntzen and

173   Sparreboom, 1989), rodent copulation (Langtimm and Dewsbury, 1991), breeding behavior of

174   three-spined sticklebacks (McLennan, 1991), nest architecture of sweat bees (Packer, 1991),

175   avian parental care (McKitrick, 1992), cooperative breeding of perching birds (Edwards and

176   Naeem, 1993), nest architecture of swallows (Winkler and Sheldon, 1993) and courtship

177   displays in birds of paradise (Scholes, 2008).

178   Phylogenetic analysis can be used to infer likely BLH states found in ancestors of

179   extant organisms by optimising or mapping characters onto an independent phylogeny

180   (McLennan, 1991). Such analyses have not progressed significantly over the last decade since

181   work by Omland (1999). There are several different approaches as characterised by Martins

182   (1999): linear parsimony (Swofford and Maddison, 1987), sum of squared changes parsimony

183 (McArdle and Rodrigo, 1994), maximum likelihood (Schluter et al., 1997), and generalised

184 least squares (Martins and Hansen, 1997). We attempt to reconstruct the mode of life and

185 appearance of the most recent common ancestor (MRCA). Current views about the MRCA

186 claim that it was a small *Eudyptula*-like diver that arose in the New Zealand region

187 (Jouventin, 1982). Stonehouse (1975) suggested that the flightless condition arose in the

188 MRCA which was no larger than the smallest living species (*Eudyptula*). In terms of its BLH

189 traits, the most recent common ancestor for the extant clade probably walked and swam in a

190 manner similar to extant penguins, was a general forager in productive shallow water habitats,

191 had few predators, and no migration (Simpson, 1976). Early penguins are thought to have

192 evolved in a broad southern zone where water temperatures (12–18°C) were warmer than

193 today (Simpson, 1975). Fordyce and Jones (1990) have reported a penguin fossil (24 million

194 years before present - mybp) morphologically similar to *Eudyptula minor*, although it is likely

195 that this is due to convergence and does not represent relatedness (Clarke et al., 2007). Baker

196 et al. (2006) suggested that the ancestor of most modern penguin groups moved out of

197 Antarctica and successfully colonised lower latitudes. In as much as behavior and life history

198 are linked to morphology we might expect some BLH traits to be as conservative over

199 penguin evolution as their morphological traits appear to be.

200   In this study we obtain behavioral and life history characters for the penguins. We test

201 the following predictions: that there is significant structure in these data and that it is largely

202 phylogenetic signal, that individual characters are good hypotheses of homology and that

203 different data types share similar levels of homology, and that ancestral states can be

204 reconstructed. An estimate for the BLH character states for the most recent common ancestor

205 will be made.

206

207 **Methods**

208 *Behavior and life history data*

209   Fifteen penguin species were analysed in this study (see Table 1). The unrepresented

210   species were *Spheniscus humboldti* (Humboldt's Penguin) and *S. mendiculus* (Galapagos

211   Penguin), which were excluded because of insufficient information (i.e. states for more than

212   half of the characters were unknown). We used the gull *Larus dominicanus* (Order

213   Charadriiformes) as the outgroup throughout. This order is thought to be relatively closely

214   related to the Order Sphenisciformes (see Sibley and Ahlquist, 1990; McKitrick, 1991;

215   Paterson et al., 1995). Behavioral information for the species was largely extracted from the

216   Handbook of Australian, New Zealand and Antarctic Birds (Marchant and Higgins, 1990).

217   This handbook is in a standardized format, with each species described by a specialist

218   researcher, and summarizes virtually all of the literature to that date. Information for all

219   species was also collected from other sources (Eggleton and Siegfried, 1979; del Hoyo et al.,

220   1992). Additional information was collected for specific species: *S. magellanicus* from

221   Stonehouse (1975), *S. demersus* from Eggleton and Siegfried (1979), *Eudyptula minor* from

222   Waas (1991), *Eudyptes pachyrhynchus* from Warham (1974) and *L. dominicanus* from

223   numerous sources (Tinbergen, 1959; Saunders, 1971; Watson, 1975; Soper, 1976; Nugent,

224   1982; Cramp, 1983; Robertson, 1985; Ehrlich et al., 1988). While datasets compiled from the

225   literature may be weaker than those derived from direct observation, we have ensured that our

226   BLH information is of high quality by going back to primary sources where possible.

227   Our choice of characters came down to two basic kinds. Traits that were explicitly

228   behavioral were those identified as stereotypical (often as fixed action patterns) displays and

229   included characters 14–25, 30–35 and 27–40. Traits that were life history related were those

230   that had measurable consequences as a result of behavioral decisions made reliably by all

231   appropriate individuals of a species. Life history traits, such as which vegetation type a nest is

232   located in, are the outcome of the interaction of several behavioral systems. They are no

233   different to measuring other behavioral outcomes such as caddis fly cases (Stuart and Currie

234   2001) or ovenbird nest architecture (Zykowski and Prum 1999). As the aim of this study was

235   to assess the overall information content of BLH characters, efforts were made to utilise all

236   available penguin BLH information. We omitted only two types of characters prior to

237   finalizing the dataset: characters that were invariant (across these taxa and a range of other

238   outgroups) and characters with unknown states in more than half of the taxa. We used the

239   principle of primary homology (Ignarsson and Coddington 2007), where homology is assumed

240   based on similarity, in order to assign our characters. We used the principle of secondary

241   homology, congruence with other phylogenetic hypotheses generated with other data, to test

242   these character states (Ignarsson and Coddington 2007).

243

244   *Phylogenetic analysis*

245   Of the 68 BLH characters (Appendix 1), 27 were multi-state and analysed as

246   unordered. Numerical character states were partitioned where there were natural breaks in

247   quantitative data. An important issue in the use of BLH characters to reconstruct phylogeny

248   concerns coding and weighting of these characters. Some authors (Felsenstein, 1983;

249   Templeton, 1983) have argued that strict parsimony, where a loss is as likely as a gain, may

250   not be the optimal coding for characters, particularly where losses are more likely than

251   independent gains. One such situation may be complex behavioral displays and actions such

252   as head circling, found in *A. forsteri* and *A. patagonicus*. This behavior features birds

253   throwing their heads back, then moving them forward in lateral, half circling motions while

254   groaning. It would seem less likely that a complex behavior of this sort would arise

255   independently than it would be lost, and should be coded as such. One way of factoring such

256   directionality is dollo parsimony (Maddison and Maddison, 2005; Cruickshank and Paterson,

257   2006), which allows convergence for character loss but assumes that a state gain can only

258   occur once. Note that dollo parsimony makes no assumption about the length of time required

259   for complex characters to evolve or be lost, only that losses are much more likely than gains.

260   A less extreme form of dealing with the problem of complex behaviors may be to weight

261 gains heavier than losses (Paterson et al., 1995). Our first analysis of the BLH data coded all

262 characters equally as unordered. A second analysis used the same characters, but designated

263 15 of them (those that we hypothesised as less likely to have arisen more than once) as dollo

264 characters (10, 15, 22, 32, 33, 37, 38, 40, 52, 53, 54, 56, 65, 66, 67). All characters were

265 assumed to be independent and were weighted equally. It is probable that some characters

266 have a more rapid rate of evolutionary change than others but there were no reasons *a priori*

267 for selecting differential weighting (see Eernisse et al., 1992). The independence of characters

268 was assessed by ensuring that all character states were mutually exclusive within and between

269 characters. Just as 'organisms are hierarchies of parts' (McKitrick, 1994), we view complex

270 behaviors as hierarchies of simple behaviors and, moreover, as diagnostic features for

271 underlying neural structures and organization (see Japyassu and Machado, 2010). For

272 example, a complex behaviour, such as nest building, was considered to be a suite of

273 phylogenetically independent characters, i.e., made up of several less complex behaviors with

274 their own individual, but congruent, evolutionary history.

275   The data were analysed using the branch and bound option of PAUP* 4.0b10

276 (Swofford, 2002) to generate BLH cladograms. The most fundamental question that can be

277 asked about the data derived from the penguin BLH dataset is whether it contains any more

278 cladistic information than a random dataset. To answer this question we employed a

279 permutation tail probability (PTP) test (Faith, 1991; Faith and Cranston, 1991) in PAUP* to

280 test for significant cladistic structure and used the g_1 statistic to evaluate the skew in the tree

281 length distributions. The PTP test shows whether the structure in the most parsimonious tree

282 could have arisen by chance alone. This test randomises character states without replacement

283 across taxa for each character and derives the shortest tree-length for the randomised dataset.

284 Tree-length reflects the degree to which shared characters co-vary upon a tree for a given

285 character set. A value for the cladistic covariation was obtained by comparing the length of

286 the most parsimonious tree with shortest tree-lengths derived from randomised datasets. If the

287   tree-length of the BLH shortest tree is not significantly smaller than shortest trees from the

288   same dataset after randomisation then BLH data contains no significant phylogenetic

289   structure. This study used 10000 randomised datasets to test for phylogenetic structure.

290   We assessed whether the incongruence between the BLH trees and the other estimates

291   of penguin phylogeny (Fig. 1) could be due to sampling error or noise in the data by using the

292   KH test (Kishino and Hasegawa, 1989) in PAUP*. We used the SH test (Shimodaira and

293   Hasegawa, 1999) implemented in PAUP* (with a resample estimated log-likelihood [RELL]

294   test distribution using 1000 bootstrap replicates) to compare the molecular tree of Baker et al.

295   (2006) with the trees generated from other datasets.

296

297   *Homology testing*

298   The homology of the BLH characters was assessed by mapping them onto the best

299   estimate of penguin phylogeny - that derived from the concatenated sequences. Individual and

300   overall measures of homoplasy, and instances of convergence, gains and losses, were

301   calculated for BLH characters by mapping them onto the concatenated sequence tree. We took

302   as our best measure of penguin phylogeny a tree derived from >5kb of mitochondrial and

303   nuclear DNA (Baker et al., 2006). Other molecular studies, e.g., Bertelli and Giannini (2005)

304   and Ksepka and Clarke (2010) provide largely similar placements of penguin taxa. This tree

305   will hereafter be described as the molecular tree.

306

307   *Display characters*

308   In order to assess relative phylogenetic value of display or behavioral (14–25, 30–35,

309   37–40) compared to non-display or life history (1–13, 26–29, 36, 41–68) characters, these

310   characters were mapped onto the molecular tree and measures of homoplasy (consistency

311   index [CI] and retention index [RI]) and numbers of gains and losses were calculated for each

312   character. Trees were constructed from display only and non-display only character datasets

13

313   using the branch and bound option of PAUP*. The trees were then compared to the molecular

314   tree.

315

316   *Character evolution*

317   The non-continuous BLH characters were optimized onto the molecular tree using a

318   parsimony approach and character states were reconstructed to determine the likely states

319   present in the most recent common ancestor of extant penguins (MRCA). There are several

320   methods by which characters are optimized onto trees and ancestors reconstructed. The most

321   appropriate method of optimising BLH characters is the Acctran option of PAUP*. The

322   Acctran option prefers reversals over parallelisms which approximates our assumption that

323   complex behaviors are unlikely to continue to evolve in concert in different species. Retention

324   indices were calculated for each character (when mapped onto the molecular tree), and these

325   were compared for display versus non-display characters.

326

327   **Results**

328   *Phylogenetic analysis*

329   Parsimony analysis of the 68 unordered BLH characters (Table 1, Appendix 1)

330   produced two shortest trees (Fig. 2; TL = 190, CI = 0.56 and RI = 0.59). Parsimony analysis of

331   the same BLH dataset using dollo parsimony for 15 of the characters produced a single most-

332   parsimonious tree (Fig. 3, TL = 196, CI = 0.54, RI = 0.67). The dollo tree differed from both

333   unordered trees (although not significantly). For example, both unordered trees had

334   *Megadyptes antipodes* as sister to a clade comprising the black-footed penguin together with

335   all *Pygoscelis* and *Eudyptes*, whereas *M. antipodes* was placed within *Pygoscelis* in the dollo

336   analysis.

337   The PTP tests (for both unordered and dollo parsimony) revealed that penguin BLH

338   data contained significant cladistic structure ($P = 0.0001$), i.e., the shortest trees were

14

339   substantially shorter than all of the trees generated by randomising the data. Similarly, the

340   significantly skewed tree length distributions ($g_1 = -0.911$ for unordered parsimony and $g_1 = -$

341   0.629 for dollo parsimony from 10000 random trees, $P < 0.01$; Hillis and Huelsenbeck, 1992)

342   indicates that the data contain significant signal. The BLH trees were generally incongruent

343   with previous estimates of penguin phylogeny (Fig. 1), with some exceptions. Estimates were

344   not significantly different for Jouventin, O'Hara, or Sibley and Ahlquist for the unordered

345   data (Table 2); or for Livezey (external morphology), O'Hara, or Sibley and Ahlquist for the

346   dollo data (Table 3). The SH tests showed that the molecular data could reject the BLH tree

347   topologies and the other alternatives to the molecular tree, except that of Sibley and Ahlquist

348   (Table 4).

349

350   *Homology testing*

351   The BLH characters were mapped onto the molecular tree. Assessing the covariance of

352   characters onto the trees found seventeen characters (2, 9, 11, 13, 17, 19, 21, 25, 29, 34, 37,

353   38, 43, 47, 50, 65, 67) that had RIs of ≥ 0.80, i.e. fitted well. Ten characters (4, 10, 14, 20, 23,

354   26, 32, 33, 61, 64) showed a total lack of fit (RI = 0.00), one (27) with an RI of 0.17, and one

355   (44) with an RI of 0.20. Nine characters (3, 5, 6, 28, 53, 55, 56, 57, 62) were autapomorphic,

356   making their RIs irrelevant.

357

358   *Display characters*

359   Mapping of display and non-display characters onto the molecular tree indicated that

360   they had similar levels of homoplasy (see Fig. 4), i.e. the difference between the RIs was not

361   significant (Mann-Whitney, $U = 373.5$, df = 1, $P = 0.599$). This finding suggests that penguin

362   display characters are no less homoplasious than the other BLH characters.

363   Parsimony analysis of the display characters alone produced 268 shortest trees (Fig. 5;

364   TL = 56, CI = 0.66, RI = 0.70). Parsimony analysis of the non-display characters found 297

365   shortest trees (Fig. 6; TL = 130, CI = 0.53, RI = 0.569). The Kishino-Hasegawa tests indicated

366   that the display dataset could reject, whereas the non-display dataset could not reject (just), the

367   molecular tree (display: Ldiff = 6, t = 2.3238, P (one tailed) = 0.0152; non-display: Ldiff = 8, t

368   = 1.6641, P (one-tailed) = 0.0515).

369

370   *Character evolution*

371   The distribution of BLH traits estimated for the MRCA are hypothesized as a pursuit

372   diver that used crustaceans and/or fish as food, capturing food in short local trips in groups.

373   The monogamous MRCA was a hillside nester, probably in arid conditions, had only a local

374   migration phase, and bred in summer with the male contributing to nest and chick duties.

375   Chicks were semi-altrical, nidicolous and creched soon after birth. Moults were complete and

376   occurred in late summer. It performed few current reproductive displays, other than ecstatic

377   and mutual bowing, and few current agonistic displays, except for primarily aggressive

378   behaviors such as bite-nape fighting. Similar results were obtained if alternative outgroups of

379   southern seabirds, Procellariiformes (*Pterodroma inexpectata*) and Pelecaniformes

380   (*Stictocarbo punctatus*), were used.

381

382   **Discussion**

383   Our phylogenetic analyses indicate that penguin behavior and life history traits contain

384   only limited phylogenetic information, unlike the results of several other similar studies of

385   different taxa (e.g., McLennan et al., 1988; Arntzen and Sparreboom, 1989; Prum, 1990;

386   Paterson et al., 1995; Kennedy et al., 1996; Slikas, 1998; Noll, 2002; Stuart et al., 2002). The

387   PTP test and g_1 statistics revealed that BLH characters contained more structure than expected

388   due to chance, but the KH and SH tests showed that this signal was significantly different

389   from the presumed correct molecular phylogeny derived from several gene regions. When

390   trees from independent datasets are congruent it is assumed that the congruence is caused by a

391   common phylogenetic signal contained within each (Penny et al., 1982; Zink and Avise,

392   1990). However, the KH tests were significant ($P = 0.0432$ and 0.0291, thus the BLH data can

393   reject the molecular tree topology) as are the SH tests (all <0.001, thus the molecular data can

394   rejects the BLH tree topologies), all of which implies that there is not a great deal in common

395   between the BLH and molecular trees. Therefore, the structure found in the BLH data is not

396   necessarily generated by phylogeny. This is readily apparent from even a cursory inspection of

397   the BLH trees. In the unordered, dollo, display and non-display trees (Figs. 2–3, 5–6) only the

398   position of *Aptenodytes* as sister to the rest of the penguins and the monophyly of *Eudyptes* is

399   the same as the molecular tree. The sister relationship of *Aptenodytes* agreed with Schreiweis,

400   Jouventin, Livezey, and Sibley and Ahlquist. Both unordered and dollo trees found that

401   *Pygoscelis* and *Spheniscus* were polyphyletic, with Black-footed (*S. demersus*) as sister taxon

402   to Yellow-eyed (*M. antipodes*) in the dollo tree. The unordered trees agreed with the

403   molecular tree in the placement of most of the *Eudyptes* taxa. Only the placement of the Erect-

404   crested (*E. sclateri*) within the genus differed from the molecular tree for one of the BLH

405   trees. The dollo tree only found the Macaroni (*E. chrysolophus*) and Royal (*E. schlegeli*) pair

406   within *Eudyptes*. There is no evidence from the other datasets that the pygoscelid penguins are

407   a paraphyletic group.

408   Why is there a minimal amount of phylogenetic information in the BLH dataset? The

409   display characters showed similar levels of homoplasy to the non-display characters, and, if

410   anything, the resulting non-display based phylogeny was marginally less inaccurate (given that

411   it was only just significantly different from the molecular phylogeny) than the display based

412   phylogeny. Adaptation is particularly effective at erasing traces of phylogeny. It may be that

413   penguin behavior and life history is itself severely constrained by the constraints of the marine

414   environment (and changes in morphology to survive there) and that character states often

415   represent convergence and parallelisms rather than homology. Such convergence has been

416   observed in behavioral calls of lacewings probably because of the constraints on methods to

17

417   actually produce sounds (Henry et al., 1999). Another issue is that of our homology

418   hypotheses. Although all of our characters satisfied primary homology requirements

419   (characters were similar) and most satisfied secondary homology requirements (character

420   states were congruent with an accepted phylogeny), there are difficulties in coding

421   behavioural character states because there are multiple hypotheses that can be encoded. For

422   example, Ksepka and Clarke (2010) have five reproductive characters in their data set that are

423   similar to ours. However, where Ksepka and Clarke have a character for clutch size and one

424   for size of first egg to second, we have one character where all of this information in encoded

425   into multiple states. Ksepka and Clarke (2010) have one character on nest information

426   whereas we have three. Ksepka and Clark (2010) use the ecstatic display as either present or

427   absent whereas we have six different states. None of these differences are contradictory but

428   they do represent different hierarchical views on the primary homology of penguin behavioral

429   traits.

430

431   *Homology testing*

432   Studies of behavioral characters have shown that these characters may be no more

433   homoplasious than other types of characters (de Queiroz and Wimberger, 1993; Paterson et

434   al., 1995). We assumed this to be true of the BLH characters used in this study. In general this

435   assumption was supported by the overall distribution of individual RIs. For instance, there

436   were more characters that fitted the molecular tree well (17 with RI ≥ 0.80) than those that

437   fitted poorly (12 with RI ≤ 0.20, see Fig. 4), although these 12 introduced considerable

438   homoplasy to estimates of phylogeny. Levels of homoplasy in non-display relative to display

439   characters were similar. A more direct test for the homology of individual characters is to

440   assess whether the behavior has arisen only once. Each display character was mapped on the

441   molecular tree and numbers of gains and losses of each character were derived under two

442   scenarios (Table 5). First, gains and losses were assumed to be equivalent in cost and, second,

18

443   independent gains were thought to be much less likely than independent losses (dollo).

444   Thirteen characters appear to have clearly arisen once. For example, treading (34) arose deep

445   in the lineage and has been retained by all descendent taxa (Fig. 7a). Seven display characters

446   (14, 16, 18, 20, 25, 31, 40) may have arisen twice if gains and losses are considered equally

447   likely and two (24, 35) may have arisen three times. Each of these nine characters was

448   reassessed to calculate the likely number of losses implied if the display had arisen only once

449   (Table 5). For example, the stare behavior (character 18) appears to have arisen twice: in *A.*

450   *patagonicus* and in the ancestor of the other penguin genera. If this behavior is assumed to

451   have arisen only once then a loss in the *A. forsteri* branch is required to explain the observed

452   pattern (Fig. 7b). Only three characters (25: bill hiding, 31: allopreening, and 35: head shake

453   and whine) posit fewer evolutionary events by assuming that the characters are not

454   homologous. For example, bill-hiding (25) may have either evolved twice, once in the

455   *Eudyptes* and once in the *Spheniscus* (two events, Fig. 7c), or once deeper in the tree but then

456   lost once in *Eudyptula* and once in *Megadyptes* (three events). These BLH characters may

457   represent the type of traits that Tinbergen (1959) hypothesized to be similar "because of the

458   limited number of possibilities of doing the opposite".

459   Stability of behavior over evolutionary time is vital for behavioral characters to be

460   useful in the construction of phylogeny. Penguins have a fossil history of more than 60 mybp

461   (Slack et al., 2006). BLH characters appear to have persisted in penguin lineages over

462   considerable evolutionary periods, e.g., pursuit diving (character 3: state 1), stare (18: 1), bite

463   nape fight (20: 1), ecstatic (30: 1-5), mutual bowing (32: 1), treading (34: 1), and nesting

464   behavior (54: 1). The most parsimonious inference is that many behaviors have persisted since

465   the origin of the extant penguin species, i.e. the behavioral characters have persisted for at

466   least 13 million years (Ksepka and Clarke, 2010) or possibly as much as 30 million years

467   (Baker et al., 2006). Ethologists have often assumed that behaviors can be simply divided into

468   those that are "innate" and those that are "learnt". Developmental researchers have argued that

469   this division is simplistic and misleading (Lehrman, 1953; Gray, 1992). One assumption that

470   is typically thought to follow is that only behaviors considered to be "innate" contain

471   phylogenetic information. This would exclude many behaviors that may have phylogenetic

472   value. What is important for the persistence of behavioral characters down lineages is not how

473   the behavior develops but that the conditions necessary for the development of the behavior

474   reliably reoccur through the phylogenetic history of organisms (Gray, 1989; Gray, 1992).

475   Many features of the penguin's physical (e.g. marine habitat, constant temperature, salinity)

476   and social (e.g. predators, competitors, colonial nesting) environment, and morphology (e.g.

477   flippers, monotypic feathers) have remained unaltered over millions of years. The persistence

478   of these features may lead to repeated development of phylogenetically informative behavioral

479   characters.

480

481   *Character evolution*

482   By examining the distribution of BLH character states at the internal branch between

483   the outgroup and penguins, we reconstructed the likely BLH traits of the MRCA. The penguin

484   ancestor has been claimed to be either a member of the genus *Eudyptula* or at least

485   eudyptulid-like and inhabited the New Zealand region (Jouventin, 1982). Phylogenetic

486   evidence from the molecular tree indicated that *Eudpytula* is not the basal genus of the

487   penguins (or in any of the hypotheses of Fig.1). The Magellanic (*S. magellanicus*) and Gentoo

488   (*P. papua*) were found to have most sequence composition in common with the hypothesised

489   MRCA. Most of the shared BLH character states between the MRCA and these species are

490   those in common with the majority of the other penguin taxa. It appears unlikely, therefore,

491   that the most recent common ancestor of extant penguins was more similar, in terms of

492   behavior and life history, to the Little blue (*E. minor*) than to the other penguin species.

493   The MRCA had a similar life history to extant penguins but had few of the ritualised

494   reproductive and agonistic behaviors. This agrees with the predictions made by Simpson

495 (1975; 1976), Jouventin (1982) and Fordyce and Jones (1990). One problem with assessing

496 BLH character states of ancestors is that we obviously cannot reconstruct extinct behaviors.

497 The reconstructed MRCA appears to have had few complex displays, or if it did, they were

498 subsequently lost in lineages leading to extant species. The latter seems unlikely, as the

499 likelihood of most of the MRCA complex behaviors completely disappearing to be replaced

500 by new complex behaviors would require many evolutionary events. The idea of the

501 MRCA with fairly simple BLH traits is also consistent with several ethological views on the

502 evolution of behavior. For example, the ritualisation of behavior is the evolutionary change of

503 a behavior in a direction that exaggerates the signal delivered to the receiver (Tinbergen,

504 1959; Harper, 1991). A simple behavior may change over evolutionary time to become more

505 complex (ritualised) to reduce signal ambiguity and manipulate and deceive the receiver

506 (Harper, 1991). Alternatively, a simple behavior may become more complex over time by run-

507 away sexual selection due to a Fisherian process or because the behavior is an advertisement

508 for male quality (Kodric-Brown and Brown, 1984). All of these alternatives are consistent

509 with an ancestor possessing less complex behaviors.

510 Did the MRCA inhabit Zealandia (Trewick et al., 2007)? New Zealand, and its

511 surrounding islands, shows the greatest diversity of penguin species with five of the six

512 penguin genera breeding or migrating throughout the region. New Zealand has, thus, been

513 viewed as the likely area for origin of penguins (Jouventin, 1982; Fordyce and Jones, 1990).

514 During the key phase of extant penguin diversification, the Zealandia region was largely

515 inundated with only small island groups available for habitation (Landis et al., 2008). Baker et

516 al. (2006) have suggested that extant penguins moved out of Antarctica during this period and

517 colonised the more temperate regions. Both of the basal penguin genera, *Aptenodytes* and

518 *Pygoscelis*, currently inhabit high southern latitudes. BLH traits of the MRCA are generally

519 consistent with inhabiting this region with short summers in which to breed, (e.g. breeding in

520 summer, and nesting in environments with little or no vegetation, short nest preparation

21

521   period, creching of chicks, moulting in late summer, when there is plentiful food). In the mid

522   to late Cretaceous New Zealand occupied a more cold temperate and southerly position, 85–

523   55°S (Fleming, 1979; Stevens, 1989; Cooper and Millener, 1993). Given that the MRCA is

524   most similar in behavior and life history to the extant Magellanic (*S. magellanicus*) and

525   Gentoo (*P. papua*) and that both of these species inhabit high latitude temperate regions the

526   evidence is consistent with a hypothesis that penguins originated in the Zealandia region.

527   One particular confounding factor in this study may be the use of a single outgroup.

528   The lack of several outgroups was largely the result of a difficulty in homologising behaviors

529   in other possible bird groups. Penguins are a very distinct taxonomical group with a

530   behavioral environment that differs from other birds. Close relatives, like Procellariiformes,

531   share a common ancestor with penguins in excess of 60 million years ago (Ksepka and

532   Clarke, 2010). We attempted to obtain traits from Procellariiformes and Pelecaniformes but

533   found it difficult to make sensible homologies. Nixon and Carpenter (2012) suggest that it is

534   more sensible not to hypothesize homology when analogy is more likely. We could sensibly

535   find homologies between Charadriiformes species and the penguins but given that the origin

536   of the extant penguins may be as recent as 12 million years ago (Ksepka and Clarke, 2010)

537   there is no simple way to break the long branch to the outgroup. This is a weakness of using

538   behaviour traits and may affect the rooting of the phylogeny and other aspects of topology.

539   A parsimony approach to reconstructing ancestral states is not without its flaws

540   (Cunningham, 1999). Several other methods using maximum likelihood (Schluter et al., 1997;

541   Maddison and Maddison, 2011) and generalised least squares (Martins, 1999; 2004) are more

542   sophisticated approaches. For the most part, however, these work with continuous characters

543   which make up only a few of those presented in this study or require models of evolution for

544   traits that are currently unknown for behavior.

545   In summary, Brooks and McLennan (1991) have emphasized the productive links that

546   are possible between behavioral, ecological and phylogenetic research. Our study indicates

547   that phylogenetic methods can 1) partially reconstruct penguin phylogeny from behavioral and

548   life history data, 2) assess the homology of behavioral characters, and 3) make inferences

549   about behavioral and ecological evolution. Why are BLH characters in penguins less

550   phylogenetically informative than BLH traits in other bird groups, such as procellariiforms

551   (Paterson et al., 1995), manakins (Prum, 1990), pelecaniforms (Kennedy et al., 1996) and

552   storks (Slikas, 1998)? Homoplasy can be an indication that you have made an error in

553   homologising traits (Nixon and Carpenter, 2012). Penguin BLH traits are either more difficult

554   to operationally group into characters and states, or are more prone to convergence. Most of

555   the behavioral traits appear to have persisted for a great length of time. It may be that, like

556   morphology, much of penguin behavior and life history is subject to intense constraints from

557   the marine environment and that this adaptive force tends to obscure phylogenetic signal.

558   Identifying penguin BLH traits that are less prone to adaptive pressure from the marine

559   environment, like reproductive displays, clutch size and nest-site fidelity, may provide a

560   source of characters that contain primarily phylogenetic information.

561

567

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822

823

825 Matrix showing behavioral and life history character data for all taxa

		1	2	3	4	5	6	
		1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	12345678
828 *Larus dominicanus*	Southern black-backed gull	000000?00??0?000000000000001?000000?00000000000?000?00?000000000??0?
829 *Aptenodytes forsteri*	Emperor penguin	021010101?0200130000101000110301104111001011122111101111211230101210
830 *Aptenodytes patagonicus*	King penguin	021010111?0100130201101000110311003111001011020111001111111120101210
831 *Pygoscelis antarctica*	Chinstrap penguin	13101?00000001?1?11111110?0121?1112000??000011??141?11112112010001?1
832 *Pygoscelis adeliae*	Adelie penguin	0310100001000111011112110000?21011101001011001120020112112012201001?1
833 *Pygoscelis papua*	Gentoo penguin	0311000010000?20??1????001?2401111000120000010020?11112?321?100110
834 *Spheniscus demersus*	Black-footed penguin	121110000?20102112?11211100?221111?0001100211??1?40?1111204340?00?11
835 *Spheniscus magellanicus*	Magellanic penguin	121010?0??2110?0?????0???01?141111?100??02202??1041???11202200100111
836 *Eudyptula minor*	Little blue penguin	121210100101001111112100010?121111000000002021?00401111120321001?211
837 *Megadyptes antipodes*	Yellow-eyed penguin	12111100?000002111132000?103110110100110000211114111101201210110011
838 *Eudyptes pachyrhynchus*	Fiordland penguin	11101000??1100210111311110013111110000011200211103121112032100121
839 *Eudyptes robustus*	Snares penguin	131010?0?11100211?1131111011311111120?0001120121?10311121120200?002120
840 *Eudyptes sclateri*	Erect-crested penguin	131010?0?1?1002111113111??0?3111112?0001120100110310111120200?00?11?

34

841	*Eudyptes chrysocome*	Rockhopper penguin	13101000?10?002?11113?1111113411112000111200021?1031?111120200?002110
842	*Eudyptes chrysolophus*	Macaroni penguin	13101001010?0?2?1?1131?110013511??2?001?1000111?0310111120200?102110
843	*Eudyptes schlegeli*	Royal penguin	131?1001?10?0?211111311?10013111112?0011100111?1031?11?1202000002111

844 Missing data indicated by "?". Characters and character states are identified in Appendix 1.

845

846 Table 2

847 Results of Kishino-Hasegawa tests comparing other estimates of phylogeny of the penguins (e.g., see Fig. 1) to those derived from the BLH data

	Tree	Length	Length diff	s.d.(diff)	t	P (one tailed)	
848							
849	*16 taxon comparison*						
850	BLH-dollo	194	4	4.00000	1.0000	0.1605	NS
851	Baker et al.	200	10	5.74586	1.7404	0.0432	
852	Giannini and Bertelli	206	16	6.06433	2.6384	0.0052	
853	Jouventin	198	8	6.29617	1.2706	0.1042	NS
854	Livezey (E)	199	9	5.11801	1.7585	0.0416	
855	Livezey (S)	205	15	5.84757	2.5652	0.0063	
856	*13 taxon comparison*						
857	Schreiweis (*S.d.*)	184	9	4.25143	2.1169	0.0190	
858	Schreiweis (*S.m.*)	182	9	4.91571	1.8309	0.0358	
859	*7 taxon comparison*						
860	McKitrick (*S.d.*)	142	11	3.93036	2.7987	0.0034	
861	McKitrick (*S.m.*)	133	8	4.16094	1.9226	0.0294	

862	OHara (*S.d.*)	136	5	5.00000	1.0000	0.1605	NS
863	OHara (*S.m.*)	129	4	4.00000	1.0000	0.1604	NS
864	*6 taxon comparison*						
865	Sibley and Ahlquist (*S.d.*)	110	(best)				NS
866	Sibley and Ahlquist (*S.m.*)	108	(best)				NS

867 Length = length of tree, Diff = difference from shortest tree, s.d.(diff) = standard deviation of difference, t = Kishino-Hasegawa test score, P

868 (one-tailed) = probability that the tree topology is significantly different from the shortest tree, Livezey (E) = external morphology, Livezey (S)

869 = skeletal morphology. For the 16 taxon comparisons all taxa were included (the taxa represented in the previous estimates of phylogeny at only

870 a generic level had no more than two species, and were represented as monophyletic). For the comparisons with less than 16 taxa the genera

871 were collapsed to single exemplars. Because *Spheniscus* is not monophyletic in the BLH trees two comparisons were made. The first of these

872 comparisons (labelled *S.d.*) used *S. demersus* as the exemplar for *Spheniscus*, whereas the second comparison (labelled *S.m.*) used *S.*

873 *magellanicus* as the exemplar for *Spheniscus* in the BLH trees.

874

Table 3

Results of Kishino-Hasegawa tests comparing other estimates of phylogeny of the penguins (e.g., see Fig. 1) to that derived from the BLH data

with dollo coded characters

Tree	Length	Length diff	s.d.(diff)	t	P (one tailed)	
16 taxon comparison						
BLH-1	198	2	5.32524	0.3756	0.3542	NS
BLH-2	199	3	5.94879	0.5043	0.3079	NS
Baker et al.	211	15	7.78345	1.9272	0.0291	
Giannini and Bertelli	220	24	8.63868	2.7782	0.0036	
Jouventin	210	14	8.12955	1.7221	0.0449	
Livezey (E)	206	10	6.41360	1.5592	0.0619	NS
Livezey (S)	214	18	7.35050	2.4488	0.0085	
13 taxon comparison						
Schreiweis (*S.d.*)	194	16	6.39029	2.5038	0.0074	
Schreiweis (*S.m.*)	191	13	6.08767	2.1355	0.0182	
7 taxon comparison						

891	McKitrick (*S.d.*)	146	13	4.78025	2.7195	0.0042	
892	McKitrick (*S.m.*)	139	12	6.36220	1.8861	0.0318	
893	OHara (*S.d.*)	141	8	6.13286	1.3044	0.0983	NS
894	OHara (*S.m.*)	134	7	5.35738	1.3066	0.0979	NS
895	*6 taxon comparison*						
896	Sibley and Ahlquist (*S.d.*)	112	(best)				NS
897	Sibley and Ahlquist (*S.m.*)	110	1	3.89987	0.2564	0.3992	NS

898   Details as for Table 3.

899

900 Table 4

901 Results of Shimodaira-Hasegawa tests comparing estimates of phylogeny of the penguins (e.g., see Fig. 1) to that derived from the sequence data

902 (Baker et al., 2006)

	Tree	-ln L	Diff -ln L	*P*
903				
904	*16 taxon comparison*			
905	BLH-1	16164.48336	560.67018	<0.001
906	BLH-2	16183.14848	579.33530	<0.001
907	BLH-dollo	16246.38993	642.57675	<0.001
908	Giannini and Bertelli	15754.26199	150.44881	<0.001
909	Jouventin	15696.39594	92.58276	0.047
910	Livezey (E)	15728.82132	125.00814	0.002
911	Livezey (S)	15847.73005	243.91687	<0.001
912	*14 taxon comparison*			
913	Schreiweis	15496.07268	241.91060	<0.001
914	*7 taxon comparison*			
915	McKitrick	13052.44033	86.51622	<0.001

916	OHara	13038.41343	72.48932	<0.001

917 *6 taxon comparison*

918	Sibley and Ahlquist	12489.93162	0.00000	0.517	NS

919 Shimodaira-Hasegawa test using RELL bootstrap (one-tailed test) with 1000 bootstrap replicates.

920

921 Table 5

922 Individual CIs and RIs of behavioral display characters mapped (using ACCTRAN) onto the

923 molecular tree (Baker et al., 2006)

				(i) gains = losses		(ii) dollo	
				minimum			minimum
926	Character	CI	RI	gains	losses	gains	losses
927	14 gaping	0.50	0.00	2	0	1	1
928	15 shoulder hunching	0.67	0.67	1	0	1	0
929	16 trumpeting	0.75	0.50	2	0	1	1
930	17 bills interlocked	0.50	0.80	1	1	1	1
931	18 stare	0.67	0.50	2	0	1	1
932	19 charge	1.00	1.00	1	0	1	0
933	20 bite nape fight	0.50	0.00	2	0	1	1
934	21 hunched submissive	1.00	1.00	1	0	1	0
935	22 face away	0.40	0.40	1	1	1	1
936	23 shivering	0.33	0.00	1	2	1	2
937	24 squeal	0.33	0.50	3	0	1	2
938	25 bill hiding	0.50	0.80	2	0	1	2
939	30 ecstatic	0.71	0.50	1	0	1	0
940	31 allopreening	0.50	0.67	2	0	1	2
941	32 mutual bowing	0.50	0.00	1	1	1	1
942	33 beating wings	0.50	0.00	1	1	1	1
943	34 treading	1.00	1.00	1	0	1	0
944	35 head shake and whine	0.67	0.50	3	1	1	4
945	37 head circling	1.00	1.00	1	0	1	0

946	38	ear rubbing	1.00	1.00	1	0	1	0
947	39	stone carrying	0.25	0.50	1	3	1	3
948	40	quivering	0.67	0.75	2	1	1	2

949 The inferred number of times each character arose or was lost (ignoring multiple states in

950 multi-state characters) on the molecular tree was calculated assuming (i) gains were as likely

951 as losses (i.e., the minimal number of gains and losses combined), or (ii) dollo, where each

952 character arose only once (i.e., the minimal number of losses). In some instances more than

953 one combination of gains and losses could be inferred (i.e. give the same minimal number of

954 combined gains and losses). Thus, the two scenarios, (i) and (ii), sometimes give the same

955 minimal score.

956

957   Fig. 1. Published hypotheses of penguin relationships among their genera and among species

958   within the crested penguins, *Eudyptes* and *Pygoscelis* (i.e., trimmed to include the same set of

959   taxa as the BLH dataset). The studies and their data types are (a) Baker et al. (2006) molecular

960   tree, (b) Giannini and Bertelli (2004) integumentary and breeding and Bertelli and Giannini

961   (2005) general morphology, (c) Jouventin (1982) behavior, (d) Livezey (1989) external

962   morphology, (e) Livezey (1989) skeletal morphology, (f) McKitrick (1991) myology and

963   morphology, (g) O'Hara (1989) morphology, (h) Schreiweis (1972) myology, and (i) Sibley

964   and Ahlquist (1990) DNA-DNA hybridization.

965

966   Fig. 2. Two most parsimonious trees generated from unordered BLH data for 15 penguin

967   species and one outgroup. The bootstrap values (>50%) are shown.

968

969   Fig. 3. The single most parsimonious tree generated from BLH data with 15 out of the 68

970   characters dollo coded for 15 penguin species and one outgroup. The bootstrap values

971   (>50%) are shown.

972

973   Fig. 4. Frequency histogram of the retention index for each of the BLH characters when

974   mapped onto the molecular phylogeny (autapomorphies excluded). The grey proportion of

975   each bar indicates the display characters, whereas the black region of each bar indicates the

976   non-display characters.

977

978   Fig. 5. A majority rule consensus tree of the 268 shortest trees derived from BLH display data.

979   The bootstrap values (>50%) are shown. The percentage of 268 shortest trees that contain

980   that component are shown in italics.

981

982   Fig. 6. A majority rule consensus tree of the 297 shortest trees derived from BLH non-display

983   data. The bootstrap values (>50%) are shown. The percentage of 297 shortest trees that

984   contain that component are shown in italics.

985

986   Fig. 7. Three BLH characters (a) treading, (b) stare, (and c) bill hide mapped onto a simplified

987   penguin phylogeny. Two possible scenarios are presented for each character: independent

988   gains as likely as losses (circles = independent gains), and independent gains less likely than

989   losses (black squares = gain of character; open squares = loss of character).

990

991

Appendix 1: Behavioral and Life History Characters used in the Analysis

993   Most of the information was derived from Marchant and Higgins (1990) except *Larus*

994 *dominicanis* (*L.d.*) (Paterson et al., 1995), *S. mendiculus* (Stonehouse, 1975), and *S. demersus*

995 (Eggleton and Siegfried, 1979). Information on display characters in all penguins from

996 Eggleton and Siegfied (1979) and *Eudyptes pachyrhynchus* and *Eudyptula minor* from

997 Warham (1974) and Waas (1991) respectively. Additional information on character states was

998 obtained from *del Hoyo et al. (1992), †Costa (1991), ‡Weimerskirch et al. (1992), and

999 ¤Williams (1981a; 1981b). Where appropriate taxa are labelled by their genus and species

1000 initials (e.g., *S. demersus* as *S.d.*).

1001

1002 1.   Chicks fed each day during first weeks of nestling stage: (0) yes, (1) no.

1003 2.   Predominant food (% numbers and mass) in diet: (0) crustaceans, (1) cephalopods, (2)

1004   fish, (3) molluscs or scavenges.

1005 3.   Main method of food capture: (0) browsing, (1) pursuit diving.

1006 4.   Time of return from foraging: (0) any, (1) dusk, (2) after dark.

1007 5.   Land directly at nest-site: (0) yes, (1) no.

1008 6   No vocalizations at sea while foraging: (0) no vocalisations, (1) vocalisations.

1009 7.   Average weight of chick's meal / average weight of adult: (0) <0.1, (1) >0.1 [†*S.d.*,

1010   *Eu.m.*].

1011 8.   Adults forage inshore during breeding season: (0) yes, (1) no.

1012 9.   Time at sea foraging: (0) 24 hours or less, (1) greater than 24 hours [†all].

1013 10. Method of regurgitation: (0) complete, (1) incomplete.

1014 11. Foraging: (0) in flocks, (1) solo, (2) cooperative foraging [*all].

1015 12. Territory defended: (0) nest/burrow, (1) area around nest/burrow (2) no territory.

1016 13. Predawn chorus: (0) no, (1) yes.

1017   14.   Gaping and open yell: (0) not present, (1) neck stretched more or less horizontal, gape,
1018   carpal joints raised.

1019   15.   Shoulder hunching: (0) not present, (1) body horizontal and neck stretched to varying
1020   extent, bill horizontal or slightly up, opened or closed, carpal joints raised, call, (2) neck
1021   lowered, head and bill directed forward, shoulders raised, body leaning forward, wings
1022   held slightly forward.

1023   16.   Trumpeting: (0) bill open, directed down and forward, moves towards intruder,
1024   repetitive call not present, (1) call, bird leans forward, raises wings, steps towards
1025   intruder, (2) neck stretched in direction of intruder, bill opening and closing, call, (3) not
1026   present.

1027   17.   Bills interlocked: (0) not present, (1) birds lock bills together and wrestle.

1028   18.   Stare: (0) not present, (1) bird in upright posture, turns body and bill towards opponent,
1029   feathers sometimes erected, (2) bird faces opponent, wings raised, bill straight out.

1030   19.   Charge: (0) not present, (1) defending bird moves towards opponent with short run,
1031   upright head forward, crown erect, wings raised.

1032   20.   Bite nape fight: (0) not present, (1) bill strikes or nips feathers and skin of opponents
1033   head and neck, swatting of opponent with wing.

1034   21.   Hunched submissive: (0) not present, (1) body stretched up, neck elongated, wings held
1035   out from body, feathers sleeked, (2) body and head held low, wings held close to sides,
1036   steps, (3) body and head held low, wings extended, feathers sleeked, steps.

1037   22.   Face away: (0) not present, (1) bird looks obliquely at owners of other territories, wings
1038   forward, bill upwards and to one side, (2) body and neck held upright, wings forward,
1039   bill hung down parallel to neck, head may turn to one side.

1040   23.   Shivering: (0) not present, (1) wings and sometimes head vibrate rapidly in agonistic
1041   situations.

1042   24.   Squeal: (0) not present, (1) bird utters high pitched squeal in response to sudden danger.

1043   25.   Bill hiding: (0) not present, (1) female crouches over nest and lowers bill until hidden

1044   under body.

1045   26.   Nest /burrow-site chosen by: (0) male, (1) either.

1046   27. Average length of incubating shifts, male : female: (0) equal, (1) male longer shift.

1047   28. Males present during laying: (0) no, (1) yes [‡*A.p.*].

1048   29.   Method by which nest/burrow constructed: (0) plant and earth material patted onto nest

1049   wall, (1) burrow dug using bill and feet, (2) adult (generally male) lying in nest scrape

1050   kicks out of bowl, shifting material to the rim, (3) adult (generally female) squats with

1051   wings extended and resting on ground, pressing back with feet and rotates.

1052   30.   Ecstatic: (0) not present, (1) bird stands on toes, wings held stiffly forward, head and

1053   open bill held vertical, loud trilling call, (2) bird stands on toes, wings held stiffly

1054   forward, head and open bill held vertical, loud trilling call, head swung in arcs, (3) bird

1055   stands on toes, head and bill held vertical, followed by head waving, (4) bird stands on

1056   toes, flippers held down, head and open bill held vertical, loud trilling call, (5) bird

1057   stands on toes, flippers held down, head and open bill held vertical, loud trilling call,

1058   head swung in arcs.

1059   31.   Allopreening: (0) not present, (1) neck, throat and head of mate preened.

1060   32.   Mutual bowing: (0) not present, (1) members of pair direct open bills downwards,

1061   usually into nest-bowls, and call.

1062   33.   Beating wings: (0) not present, (1) male wings vibrated gently on females sides prior to

1063   copulation.

1064   34.   Treading: (0) not present, (1) male treads on back of female prior to copulation.

1065   35.   Head shake and whine: (0) not present, (1) head rapidly waved from side to side with

1066   bill closed and pointing at other bird, stands on toes with erect fanned tail, whining call,

1067   wings stretched, (2) rub heads together, calling, (3) birds face each other and head

1068   waved side to side, punctuated by short calls. (4) birds face each other slowing raising

1069   while contracting neck muscles.

1070   36.   Copulation outside nest/burrow: (0) no, (1) yes.

1071   37.   Head circling: (0) not present, (1) head thrown back, and then moved forward in lateral,

1072   half circular motions, while uttering groans.

1073   38.   Ear rubbing: (0) not present, (1) birds rub ear against shoulder.

1074   39.   Stone carrying: (0) not present, (1) stones, grass, or earth collected and placed around

1075   nest (generally male).

1076   40.   Quivering: (0) not present, (1) nesting bird vibrates bill in very small arcs as it bows

1077   over nest-bowl to deposit nest material, (2) bird opens bill and hisses as nest material is

1078   deposited.

1079   41.   Most adults remain near breeding site during year: (0) yes, (1) no.

1080   42.   Type of migration used by species during life cycle: (0) coastal or no migration, (1) pack

1081   ice region, (2) north of Antarctic convergence.

1082   43.   Nester or burrower (nesting in burrows): (0) nest, (1) neither, (2) either.

1083   44.   Predominant burrow/nest-site terrain: (0) cliff, (1) flat.

1084   45.   Vegetation present at nest/burrow site: (0) no vegetation, (1) tussock or grassland, (2)

1085   forest.

1086   46.   Pair-bond duration: (0) several breeding seasons, (1) lifelong, (2) one breeding season.

1087   47.   Sex ratio in adult life: (0) equal, (1) more males, (2) more females.

1088   48.   Chicks sometimes abandoned to die by parents: (0) yes, (1) no.

1089   49.   Nest-site fidelity: (0) yes, (1) no [¤*P.p.*, ¤*E.c.*].

1090   50.   Clutch size: (0) more than two, survival approximately equal for each egg/chick, (1)

1091   one, (2) two, survival of first egg/chick better, (3) two, survival of second egg/chick

1092   better, (4) two, survival approximately equal for each egg/chick.

1093   51.   Replacement laying of eggs in same season as failure of first nest: (0) yes, (1) no.

1094   52.   Sexual differences in voice: (0) yes, (1) no.

1095   53.   Nestling state of development: (0) altrical, (1) semi-altrical.

1096   54.   Nestling behavior: 0 semi-nidicolous, (1) nidicolous, (2) nudifuguous.

1097   55.   Average fledging weight/average adult weight: (0) >1, (1) <1 [†*S.d*, †*E.c.*].

1098   56.   Moult halts foraging: (0) no, (1) yes.

1099   57.   General foraging habitat: (0) marine, (1) pelagic, (2) intertidal.

1100   58.   Adults leave young before fledging: (0) no, (1) yes.

1101   59.   Season that breeding commences: (0) spring, (1) summer, (2) autumn, (3) winter (4) no

1102   set time.

1103   60.   Month of main moult: (0) September, (1) November, December, (2) February, (3) no set

1104   time.

1105   61.   Season of nest construction: (0) winter, (1) spring, (2) summer, (3) autumn, (4) no set

1106   time.

1107   62.   Ratio of age first breeding/first return to breeding colonies: (0) ~1, (1) 2+.

1108   63.   Ratio of nest preparation period/incubation length: (0) >0.66, (1) <0.66 [¤*L.d.*].

1109   64.   Ratio of nestling period/incubation length: (0) approx equal, (1) incubation period

1110   shorter than nestling.

1111   65.   Laying interval between eggs: (0) less than four days, (1) no laying interval, (2) four

1112   days or more [¤all].

1113   66.   Age of creching: (0) no creching, (1) less than 28 days, (2) 28 days or more [*all].

1114   67.   Length of premoult forage: (0) no premoult forage, (1) 3-5 weeks, (2) >5 weeks.

1115   68.   Chicks fledged/pair: (0) 0-0.9 [*all], (1) 1.0-1.9.

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