

Short communication

Canary Island Ravens *Corvus corax tingitanus* have distinct mtDNA

JASON M. BAKER† & KEVIN E. OMLAND*

Department of Biological Sciences, University of
Maryland, Baltimore County, 1000 Hilltop Circle,
Baltimore, MD 21250, USA

The Common Raven *Corvus corax* (Linnaeus 1758) breeds throughout most of the Northern Hemisphere and is the most widespread of all corvids (Madge & Burn 1994). There is little discrete morphological variation in the species, although eight races have been described (Vaurie 1959). Recent phylogenetic studies of the *Corvus corax* complex suggest that the Common Raven shows species-level paraphyly and comprises two deep evolutionary lineages, the 'Holarctic clade' and the 'California clade' (Omland *et al.* 2000, Feldman & Omland 2005). In the present study, we sought to elucidate further the phylogenetics of the 'Holarctic clade' by examining an additional *C. corax* race: *C. c. tingitanus* (Irby 1874), the smallest subspecies of ravens. It is restricted to the Canary Islands and Morocco and can be distinguished by its size and 'oily' plumage gloss (Madge & Burn 1994). This study focuses on the island populations, which we term the 'Canary Island Raven'. The geographical isolation and distinctive morphology of this group make it crucial to our goal of further assessing the extent of genetic variation within the 'Holarctic clade'.

METHODS

We obtained tissue from 27 Common Ravens: 11 from the Canary Islands and 16 from mainland Europe. We amplified 314 base pairs of the mitochondrial (mt) control region (CR) using primers corII-LGL2 and cor-H417 (see Omland *et al.* 2000). We followed standard amplification and sequencing protocols (detailed in Omland *et al.* 2000), and we deposited all new CR sequences into GenBank under accession numbers AY710328–AY710354. We used published CR sequences from an additional 26 Common Ravens (one of each unique haplotype in the 'Holarctic clade' and five total in the 'California clade'; Omland *et al.* 2000). We included published sequences from several

*Corresponding author.
Email: omland@umbc.edu

†Present address: Department of History & Philosophy of
Science, Indiana University, 1101 East Third Street, Bloomington,
IN 47405, USA.
Email: bakerjm@indiana.edu.

closely related raven species: Chihuahuan Ravens *C. cryptoleucus*, and two African taxa: Pied Crow *C. albus* and White-necked Raven *C. albicollis* (Feldman & Omland 2005). Finally, we used three other published *Corvus* sequences as outgroups: American Crow *C. brachyrhynchos*, Hawaiian Crow *C. hawaiiensis* and Mariana Crow *C. kubaryi* (see Appendix I for details).

We conducted phylogenetic analyses using neighbour-joining (NJ), maximum-parsimony (MP), and maximum-likelihood (ML) methods in PAUP* 4.0b10 (Swofford 2002). We conducted MP searches using the heuristic search algorithm with 1000 random additions. There were no insertions or deletions in the sequence data, and all characters were equally weighted. We conducted bootstrap analyses with 'max trees' set at 100, three random addition replicates and 500 bootstrap pseudo-replicates. Hierarchical likelihood ratio tests in Modeltest 3.06 (Posada & Crandall 1998) determined that the 'HKY85 + gamma' model (Hasegawa *et al.* 1985) best fits our data. We used the heuristic search algorithm to construct the ML tree, simultaneously estimating tree topology and parameter values. We successively re-estimated tree parameters and searched for trees until a stable topology and ML score were found (Wilgenbusch & de Queiroz 2000).

To test for population structure within the 'Holarctic clade', we performed hierarchical analyses of molecular variance (AMOVA) (Excoffier *et al.* 1992). We included haplotype frequency data for imported sequences to ensure representative sampling. We divided the 'Holarctic clade' into three groups: 'Canary Island' ($n = 11$), 'Italy' ($n = 13$) and 'Rest' ($n = 24$, representing 37 individuals). We used Arlequin 2.0 (Schneider *et al.* 2000) to calculate pairwise fixation indices (Φ_{ST} values, an analogue of F_{ST} that accounts for genetic distance). Extensive sampling in Italy enabled us to compare local differentiation within mainland Eurasia with differentiation between Canary Island Ravens and Common Ravens from the rest of the 'Holarctic clade'.

RESULTS

Of the 314 nucleotides in total, 90 are variable and 57 are parsimony-informative. The 'Holarctic clade' has an average uncorrected genetic divergence of 1.99% (range 0–4.47%); excluding Canary Island Ravens, the average divergence within the clade is 1.47% (range 0–3.82%). Common Ravens from Italy have an average 1.20% divergence (range 0–2.87%), and they differ from the rest of the clade, excluding Canary Island Ravens, by an average 1.60% (range 0–3.82%). By contrast, Canary Island Ravens are only 0.17% divergent on average (range 0–0.32%); they differ from the rest of the clade by an average 3.34% (range 2.23–4.47%). These percentages correspond to the following nucleotide diversity values: Canary Island, -0.0017 ; Italy, -0.0120 ; Holarctic (excluding Canary), -0.0147 (π as calculated in Arlequin).

MP searches produced 100 equally parsimonious trees of 176 steps each. Bootstrap analysis strongly supports the

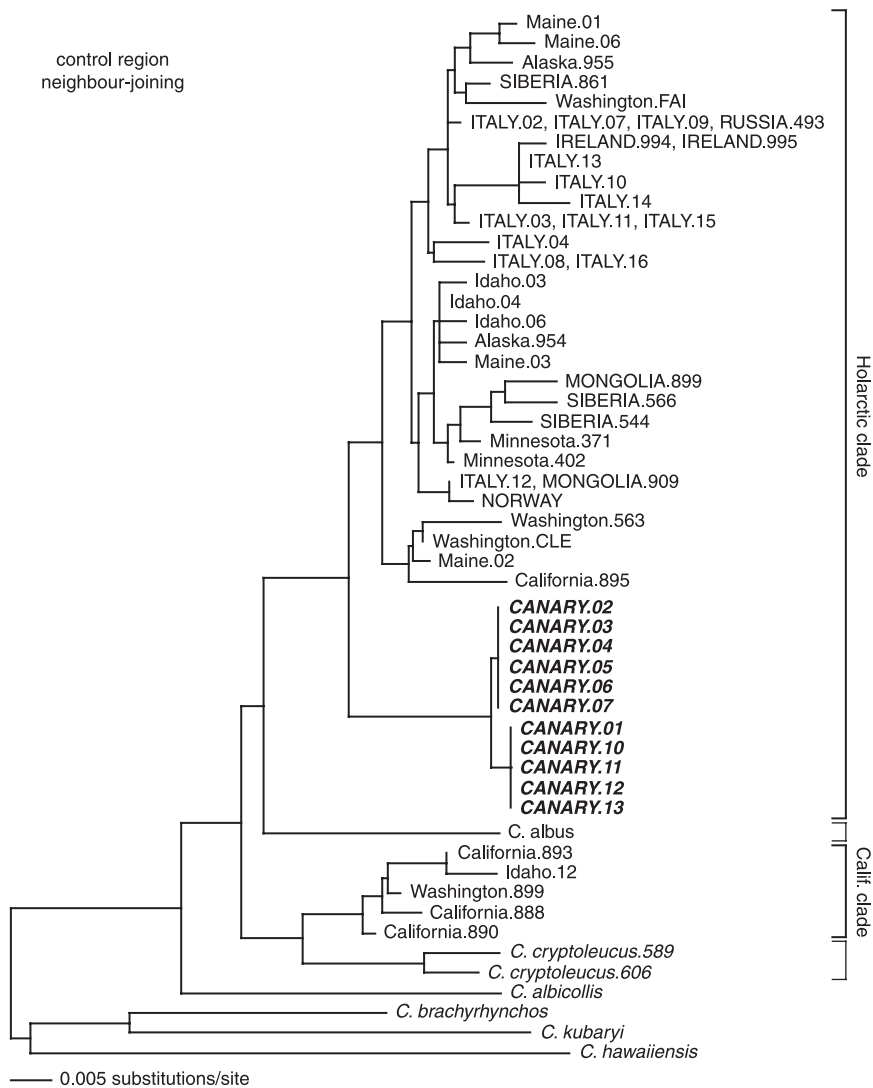


Figure 1. Neighbour-joining phylogram of the Common Raven *Corvus corax* and close relatives, based on 314 bp of the mitochondrial control region. Genetic distances are uncorrected. Canary Island Ravens are highlighted in bold. Note that in this tree Canary Island Ravens are sister to the rest of the ‘Holarctic clade’. (See Appendix for locality information.)

Canary Island Raven clade with a score of 95%. ML searches produced a single tree ($-\ln l = 1337.9690$) with the following parameter values: $ti/tv = 5.9529$, $\alpha = 0.2335$, $A = 0.3468$, $C = 0.3183$, $G = 0.1136$ and $T = 0.2213$. All phylogenetic analyses show Canary Island Ravens to be monophyletic, but they differ in the placement of the group with respect to the rest of the ‘Holarctic clade’. NJ indicated that Canary Island Ravens are sister to the rest of the clade (see Fig. 1) and ML that Canary Island Ravens are nested within the clade (see Fig. 2); MP could not resolve the relationship.

AMOVA revealed significant genetic differentiation between Canary Island Ravens and the rest of the ‘Holarctic clade’ ($\phi_{ST} = 0.6648$, $P < 0.00001$), which strongly suggests there is little or no gene flow between the groups.

By contrast, there is little differentiation between samples from Italy and samples from the rest of the clade, excluding the Canary Islands ($\phi_{ST} = 0.1787$, $P = 0.0001$).

DISCUSSION

Canary Island Ravens have mtDNA sequences that are diagnosably distinct from those of Common Ravens from the rest of the ‘Holarctic clade’. The groups differ by an average 3.34% in CR sequence. Phylogenetic analyses further suggest that Canary Island Ravens have been evolving independently of the rest of the clade. There is no haplotype sharing between the groups, and a long branch separates them (7 bp on the ML tree, for example). The groups may be reciprocally monophyletic. Population genetic

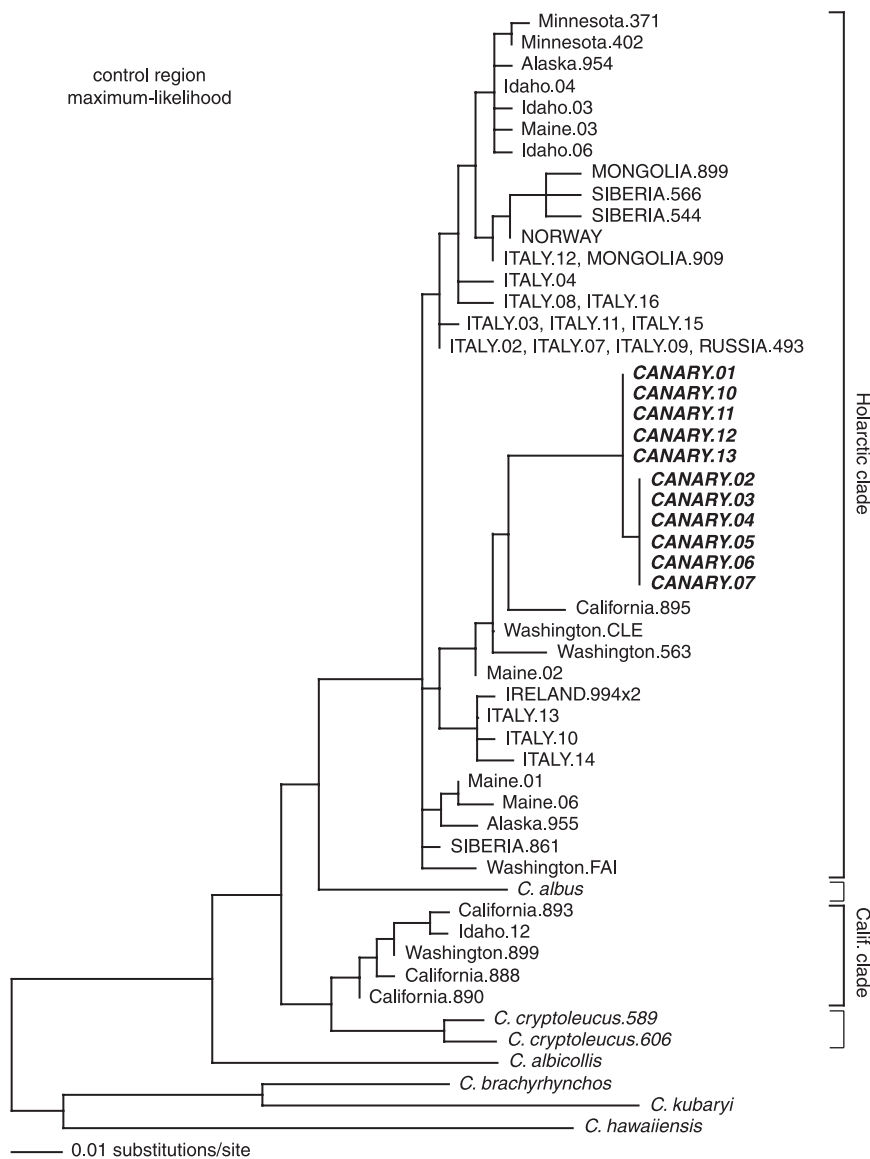


Figure 2. Maximum-likelihood phylogram of the Common Raven *Corvus corax* and close relatives, based on 314 bp of the mitochondrial control region. Canary Island Ravens are highlighted in bold. Note the long branch (7 bp) that separates Canary Island Ravens from the rest of the 'Holarctic clade'. (See Appendix 1 for locality information.)

analysis strongly suggests that Canary Island Ravens are not exchanging genes with Common Ravens from the rest of the 'Holarctic clade'. Those patterns indicate a recent split between the two groups. In addition, the groups differ in several morphological characters (see Vaurie 1959, Madge & Burn 1994), including plumage coloration (Irby 1874), the presence or absence of an 'oily' plumage gloss (Niethammer 1953), wing length (Hartert & Kleinschmidt 1901) and perhaps skeletal dimensions (Hernández *et al.* 1993). Canary Island Ravens are diagnosably distinct in both mtDNA and morphology from the rest of the 'Holarctic clade'. Our findings, combined with previous

molecular studies of birds and other taxa on the Canary Islands (e.g. Marshall & Baker 1999; reviewed in Juan *et al.* 2000), provide an increasingly detailed picture of the biogeographical history of these islands.

According to criteria suggested by Moritz (1994a), Canary Island Ravens probably comprise an evolutionarily significant unit – and certainly a management unit (Moritz 1994b; see also Avise 2000). The group is demographically independent: if overexploited or extirpated, because of low gene flow with mainland ravens it is unlikely to recover (Avise 1995). In addition, the group is probably evolutionarily independent, although reciprocal monophyly with

the rest of the 'Holarctic clade' has not been demonstrated. Until additional *C. c. tingitanus* samples can be collected (from other Canary Islands and especially North Africa), we do not recommend any taxonomic revisions. Nevertheless, the clear differences we observed in mtDNA sequences between Canary Island Ravens from Fuerteventura and Common Ravens from the rest of the 'Holarctic clade' provide a strong basis for additional mainland and island sequencing and taxonomic work.

Manuel J. de la Riva-Pérez (Estación Biología de Doñana, Sevilla, Spain) provided the Canary Island Raven tissues. We are very grateful for his assistance. Thanks also to Violeta Muñoz and Jeff Peters. Many other individuals and institutions have provided tissue for this and previous work on ravens. Here, we especially thank Mareike Stöwe (Konrad Lorenz Forschungsstelle, Austria), Marianne Imhof (Veterinärmedizinische Universität Wien, Austria), Jon Fjeldså (Zoologisk Museum, Statens Naturhistoriske Museum, Denmark) and Fred Sheldon (Museum of Natural Science, Louisiana State University, USA). We thank Beatrice Kondo for translation assistance. K.E.O. was supported by the National Science Foundation.

REFERENCES

- Avise, J.C.** 1995. Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. *Conserv. Biol.* **9**: 686–690.
- Avise, J.C.** 2000. *Phylogeography: the History and Formation of Species*. Cambridge, MA: Harvard University Press.
- Excoffier, L., Smouse, P.E. & Quattro, J.M.** 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction region. *Genetics* **131**: 479–491.
- Feldman, C.R. & Omland, K.E.** 2005. Phylogenetics of the Common Raven complex (*Corvus*: Corvidae) and the utility of ND4 and COI in avian molecular systematics. *Zool. Scripta* **34**: 145–156.
- Hartert, E. & Kleinschmidt, O.** 1901. Verzeichniss der Brehmschen Sammlung. I, Die Formen von *Corvus corax*. *Novit. Zool.* **8**: 40–48.
- Hasegawa, M., Kishino, K. & Yano, T.** 1985. Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **21**: 160–174.
- Hernández, E.C., Martín, M. & Rando, J.C.** 1993. Estudio osteológico comparado de dos subespecies de *Corvus corax* (Aves: Passeriformes). *Archeoofauna* **2**: 181–190.
- Irby, H.** 1874. Notice of an apparently undescribed species of *Corvus* from Tangier. *Ibis* (3) **4**: 264–266.
- Juan, C., Emerson, B.C., Oromí, P. & Hewitt, G.M.** 2000. Colonization and diversification: towards a phylogenetic synthesis for the Canary Islands. *Trends Ecol. Evol.* **15**: 104–109.
- Linnaeus, C.** 1758. *Corvus corax*. In *Systema Naturae* (10th edn), Vol. 1: 105. Stockholm: L. Salvii.
- Madge, S. & Burn, H.** 1994. *Crows and Ravens*. Boston: Houghton Mifflin.
- Marshall, H.D. & Baker, A.J.** 1999. Colonization history of Atlantic island Common Chaffinches (*Fringilla coelebs*) revealed by mitochondrial DNA. *Mol. Phylogenet. Evol.* **11**: 201–212.
- Moritz, C.C.** 1994a. Defining 'evolutionarily significant units' for conservation. *Trends Ecol. Evol.* **9**: 373–375.
- Moritz, C.C.** 1994b. Applications of mitochondrial DNA analysis in conservation. *Phil. Trans. Roy. Soc. Lond. B* **349**: 113–118.
- Niethammer, G.** 1953. Der Kolkkrabe (*Corvus corax*) von Fuerteventura, ein Beitrag zur Tiergeographie der östlichen Kanaren. *Bonn. Zool. Beitr.* **4**: 73–78.
- Omland, K.E., Tarr, C.L., Boorman, W.I., Marzluff, J.M. & Fleischer, R.C.** 2000. Cryptic genetic variation and parphyly in ravens. *Proc. R. Soc. Lond. B* **267**: 2475–2484.
- Posada, D. & Crandall, K.A.** 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- Schneider, S. & Roessli, D. & Excoffier, L.** 2000. *Arlequin: a Software for Population Genetics Data Analysis*. Geneva: University of Geneva.
- Swofford, D.L.** 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (and Other Methods)*. Sunderland, MA: Sinauer Associates.
- Vaurie, C.** 1959. *The Birds of the Palearctic Fauna, 1. Passeriformes*. London: H.F. & G. Witherby.
- Wilgenbusch, J. & de Queiroz, K.** 2000. Phylogenetic relationships among the phrynosomatid sand lizards inferred from mitochondrial DNA sequences generated by heterogeneous evolutionary processes. *Syst. Biol.* **49**: 592–612.

Received 13 July 2004; revision accepted 22 September 2005.

APPENDIX I

Material used for analysis

Species*	Individual	Locality	GenBank accession no.
<i>C. corax</i> (H)	Alaska.954	USA, Alaska, Fairbanks County	AY005895†
<i>C. corax</i> (H)	Alaska.955	USA, Alaska, Fairbanks County	AY005896†
<i>C. corax</i> (H)	California.895	USA, California, San Bernardino County	AY005897†
<i>C. corax</i> (H)	Idaho.03	USA, Idaho, Ada County	AY005898†
<i>C. corax</i> (H)	Idaho.04	USA, Idaho, Ada County	AY005899†
<i>C. corax</i> (H)	Idaho.06	USA, Idaho, Ada County	AY005901†
<i>C. corax</i> (H)	IRELAND.994	Ireland, County Cork	AY710339
<i>C. corax</i> (H)	IRELAND.995	Ireland, County Cork	AY710340
<i>C. corax</i> (H)	ITALY.02	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710341

continued

APPENDIX I

Continued

Species*	Individual	Locality	GenBank accession no.
<i>C. corax</i> (H)	ITALY.03	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710342
<i>C. corax</i> (H)	ITALY.04	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710343
<i>C. corax</i> (H)	ITALY.07	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710344
<i>C. corax</i> (H)	ITALY.08	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710345
<i>C. corax</i> (H)	ITALY.09	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710346
<i>C. corax</i> (H)	ITALY.10	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710347
<i>C. corax</i> (H)	ITALY.11	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710348
<i>C. corax</i> (H)	ITALY.12	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710349
<i>C. corax</i> (H)	ITALY.13	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710350
<i>C. corax</i> (H)	ITALY.14	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710351
<i>C. corax</i> (H)	ITALY.15	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710352
<i>C. corax</i> (H)	ITALY.16	Italy, Regione Autonoma Friuli-Venezia Giulia	AY710353
<i>C. corax</i> (H)	Maine.01	USA, Maine, Franklin County	AF115305‡
<i>C. corax</i> (H)	Maine.02	USA, Maine, Franklin County	AF115306‡
<i>C. corax</i> (H)	Maine.03	USA, Maine, Franklin County	AF115307‡
<i>C. corax</i> (H)	Maine.06	USA, Maine, Franklin County	AY710427‡
<i>C. corax</i> (H)	Minnesota.371	USA, Minnesota, Pine County	AY005905†
<i>C. corax</i> (H)	Minnesota.402	USA, Minnesota, St. Louis County	AY005907†
<i>C. corax</i> (H)	MONGOLIA.899	Mongolia, Arhangay Aymag	AY005908†
<i>C. corax</i> (H)	MONGOLIA.909	Mongolia, Övörhangay Aymag	AY005909†
<i>C. corax</i> (H)	NORWAY	Norway, Nordland Fylke	AY710354
<i>C. corax</i> (H)	RUSSIA.493	Russia, Krasnodarskiy Kray	AY005914†
<i>C. corax</i> (H)	SIBERIA.544	Siberia, Tyumenskaya Oblast	AY005912†
<i>C. corax</i> (H)	SIBERIA.566	Siberia, Yamalo-Nenetskiy Avtonomnyy Okrug	AY005913†
<i>C. corax</i> (H)	SIBERIA.861	Siberia, Magadanskaya Oblast	AY005911†
<i>C. corax</i> (H)	Washington.563	USA, Washington, Jefferson County	AY005917†
<i>C. corax</i> (H)	Washington.CLE	USA, Washington, Kittitas County	AY005918†
<i>C. corax</i> (H)	Washington.FAI	USA, Washington, Clallam County	AY005919†
<i>C. corax</i> (C)	California.888	USA, California, San Bernardino County	AY005875†
<i>C. corax</i> (C)	California.890	USA, California, San Bernardino County	AY005877†
<i>C. corax</i> (C)	California.893	USA, California, San Bernardino County	AY005880†
<i>C. corax</i> (C)	Idaho.12	USA, Idaho, Ada County	AY005890†
<i>C. corax</i> (C)	Washington.899	USA, Washington, Yakima County	AY005891†
<i>C. c. tingitanus</i>	CANARY.01	Spain, Canary Islands, Fuerteventura	AY710328
<i>C. c. tingitanus</i>	CANARY.02	Spain, Canary Islands, Fuerteventura	AY710329
<i>C. c. tingitanus</i>	CANARY.03	Spain, Canary Islands, Fuerteventura	AY710330
<i>C. c. tingitanus</i>	CANARY.04	Spain, Canary Islands, Fuerteventura	AY710331
<i>C. c. tingitanus</i>	CANARY.05	Spain, Canary Islands, Fuerteventura	AY710332
<i>C. c. tingitanus</i>	CANARY.06	Spain, Canary Islands, Fuerteventura	AY710333
<i>C. c. tingitanus</i>	CANARY.07	Spain, Canary Islands, Fuerteventura	AY710334
<i>C. c. tingitanus</i>	CANARY.10	Spain, Canary Islands, Fuerteventura	AY710335
<i>C. c. tingitanus</i>	CANARY.11	Spain, Canary Islands, Fuerteventura	AY710336
<i>C. c. tingitanus</i>	CANARY.12	Spain, Canary Islands, Fuerteventura	AY710337
<i>C. c. tingitanus</i>	CANARY.13	Spain, Canary Islands, Fuerteventura	AY710338
<i>C. albicollis</i>	–	Malawi, Mulanje	AY527253‡
<i>C. albus</i>	–	Ghana (central)	AY527252‡
<i>C. brachyrhynchus</i>	–	USA, Maryland, Montgomery County	AY005869†
<i>C. cryptoleucus</i>	589	USA, New Mexico, Doña Ana County	AY005922†
<i>C. cryptoleucus</i>	606	USA, New Mexico, Doña Ana County	AY005927†
<i>C. hawaiiensis</i>	–	USA, Hawaii	AY005870†
<i>C. kubaryi</i>	–	USA, Guam	AF115296

*C, 'California clade'; H, 'Holarctic clade' (Omland *et al.* 2000).†Previously published in Omland *et al.* (2000).

‡Previously published in Feldman and Omland (2005).