

Morphological and genetic analyses reveal the first Mediterranean occurrence of American Herring Gull *Larus smithsonianus*

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We report here the first record of an American Herring Gull *Larus smithsonianus* in a Mediterranean coastal site (18 February–14 March 2019, Fuengirola, Malaga, S Spain). This gull was an adult bird and its identification was confirmed by a combination of mtDNA molecular analyses and morphological characters. This record furthermore stands as possibly the first ever adult bird of the 'Niagara' phenotype in Europe, which would indicate that it originated from the central region of USA/Canada or even from the west coast. This is the southernmost record of an adult bird of this species in the Western Palearctic and the eleventh for the Iberian Peninsula since 1991.

Key words: American Herring Gull, *Cytb*, *COI*, *gene sequence*, *mtDNA*, *vagrancy*.

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The American Herring Gull *Larus smithsonianus* is the most widespread large gull in the Nearctic (Howell & Dunn 2007). It is a common breeder in most of eastern North America but is somewhat less common further west (Olsen & Larsson 2003, Howell & Dunn 2007). Although it is mainly sedentary, part of the northern population winters in central and southern North America, and casually in Central America. It is a rare vagrant to the Western Palearctic (Olsen 2018) and as such in Europe it is considered a rarity and all sightings require verification by

the respective national rarities committees. Its taxonomic classification is as yet not totally clear. Gulls belonging to Herring Gull complex started to diverge relatively recently, about 300,000 years ago (Liebers *et al.* 2001), which is why most of these taxa are genetically very similar and difficult to identify reliably (Crochet *et al.* 2002, Collinson *et al.* 2008). In this sense, until recently, the American Herring Gull was considered to be conspecific with the European Herring Gull *L. argentatus*, with which it shares several characters (Threlfall & Jewer 1978,

Olsen & Larsson 2003, Adriaens & Mactavish 2004).

Liebers *et al.* (2004) used mtDNA sequences to establish that the Herring Gull complex is not a ring species, and that the American Herring Gull is genetically closer to the eastern Asian and Nearctic gull species than to the European ones. These authors agree with Crochet *et al.* (2002) and confirm the existence of two main phylogenetic clades resulting from the evolution of gull taxa in two different areas: the Atlantic clade includes taxa originating from the *argentatus* ancestral lineage (*argentatus*, *argenteus*, *michahellis* and other European gulls), whereas the Aralo-Caspian clade includes taxa originating from the *cachinnans* lineage (*smithsonianus*, *cachinnans* and *vegae*, among others).

Despite the small genetic differences between American and European Herring Gulls, several phylogenetic studies recommend treating the American Herring Gull as a polytypic species *L. smithsonianus* that is separate from the European taxa (Crochet 2002, Sangster *et al.* 2007, Collinson *et al.* 2008). Thus, the *L. smithsonianus* group (*L. s. smithsonianus*, *L. (s.) vegae* and *L. (s.) mongolicus*) is distributed throughout North America and northern and eastern Asia, while the *L. argentatus* group (*L. a. argentatus* and *L. a. argenteus*) is found in Europe. In light of these phylogenetic results, we consider here the American Herring Gull to be a separate species from the European Herring Gull (following Gill & Donsker 2019).

Despite the relative lack of records of the American Herring Gull in Europe, it would seem to be a regular visitor (Lonergan & Mullarney 2004). The American Herring Gull was first cited in European waters in 1937 (Gross 1940) and by the mid 2000s c. 70 specimens had been recorded in Europe, mainly in Great Britain (Olsen & Larsson 2003). As of 2017, in the Iberian Peninsula there had been nine accepted records (Gil-Velasco *et al.* 2019). However, all identifications of vagrant specimens in Europe have been made on the basis of morphological characters using existing criteria (see e.g. Dubois 1997, Lonergan & Mullarney 2004). This procedure has its limitations, especially in such a difficult complex as the Herring Gulls. Given the small but consistent genetic differences between American and European Herring Gulls, the DNA analysis of feathers, faeces or any other

body tissue offers a complementary means of differentiating these species (e.g. Dufour *et al.* 2016, García-Barcelona *et al.* 2016).

Mitochondrial DNA is widely used in molecular analyses. The hypervariable region of the mitochondrial 'control region' (CR), the 'cytochrome oxidase I' (COI) and the 'cytochrome b' (Cytb) are commonly used as molecular markers for identifying animal species and in phylogenetic studies above species level (e.g. Farias *et al.* 2001). For example, various studies have demonstrated that the COI sequence has an interspecific variation that is large enough to provide adequate correspondence between molecular identification and recognition based on the morphological characters of each species (Hebert *et al.* 2003, 2004), and so can act as a 'bar code'.

On the basis of both morphological features and molecular analysis of mtDNA fragments of the Control Region (CR), cytochrome oxidase gene subunit I (COI) and cytochrome b gene (Cytb), we report here the first record of an American Herring Gull for the Mediterranean. The record is of relevance because it is the first time that (a) an adult bird has been identified in Europe without having been seen as a (more easily identifiable) immature in previous years and (b) an American Herring Gull with phenotypic characteristics more typical of the Great Lakes Region of N America has been observed in the Western Palearctic.

Material and methods

Finding and collection of biological samples

On 18 February 2019, an unidentified Herring Gull was seen in Fuengirola harbour (Malaga, Spain), a small fishing harbour on the NW coast of the Alborán Sea. It remained in the same place for over a month and was repeatedly observed feeding on trawler discards or resting until 24 March 2019. During this time, a sufficient number of pictures were taken that could be used to discern in reasonable detail its morphological traits and plumage. The photographic equipment used was a DSLR camera Canon EOS 7D Mark II equipped with a Canon 300 mm f/4 L telephoto lens. In addition, the gull was followed

for several days to obtain biological samples for genetic analyses and three samples of faeces and one scapular feather were gathered. Faeces were collected using sterile swabs and kept dried until analysis. The feather was collected on a roof after it fell from the gull's body during preening.

DNA extraction and sequencing

Biological samples were sent to the Institute of Medical Sciences of the University of Aberdeen (Aberdeen, Scotland) and to the Evolutionary Ecology Department of the Doñana Biological Station (EBD-CSIC, Seville, Spain) for genetic analysis.

DNA was isolated from both the feather and the faecal samples, and the genetic sequences were retrieved from three mitochondrial loci via polymerase chain reactions and Sanger sequencing (See Supplementary Information for full details). Sequence from the Cytb gene and Control Region were concatenated and compared with publicly available *Larus* sequences located in Genbank to produce a Maximum Likelihood phylogenetic tree. Sequences from the barcoding region of the COI gene and Cytb were also compared to *Larus* sequences located in BoldSystem database (<http://www.boldsystems.org/>) and Genbank (<http://www.ncbi.nlm.nih.gov/>), respectively, to produce Bayesian phylogenetic reconstructions for each locus.

Results

Morphological description

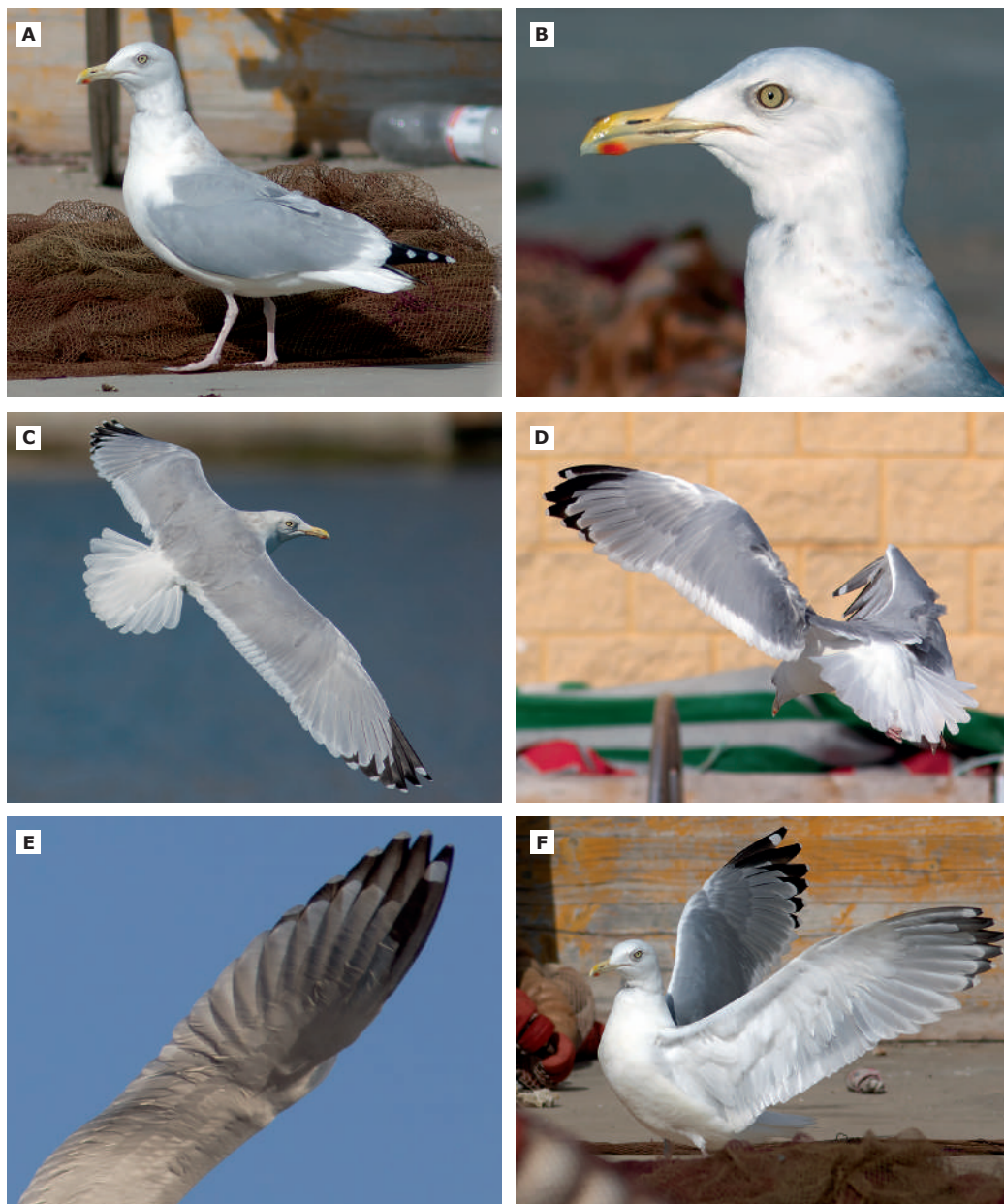
At first sight the gull drew observers' attention due to the overall paleness of its grey mantle, and its pinkish legs and pale-yellow eyes and bill. These were the most significant traits, which separated it reasonably easily from other large gull species of the same age present in the harbour: Lesser Black-backed Gull *L. fuscus* and Yellow-legged Gull *L. michahellis* (Figure 1).

This gull was stocky but similar in size to a female Yellow-legged Gull, and had light pink and seemingly short legs, and very pale-grey upperparts that were obviously paler than those of the nearest *michahellis* (Figure 2A). This important feature explains why this gull stood out from the other darker taxa present in the harbour but is something that could easily be ignored in other European countries where paler European Herring Gulls are common. At this time of year, it had already lost most of the darker winter marks on its head and neck. More features of the head and neck are shown in Figure 2B.

The outermost primaries did not have the same markings on the left and right wings (Figure 2C-D). On the left wing, P10 had a long grey tongue which reached the mirror resembling the so-called 'thayeri' pattern (after Thayer's gull *L. thayeri*, in which the medial band on the



Figure 1. Adult American Herring Gull *Larus smithsonianus* (centre, bottom) at Fuengirola harbour on 19 February 2019; top left, an adult Lesser Black-backed Gull *L. fuscus*; top right, an adult Yellow-legged Gull *L. michahellis*. Gavià argentat americà *Larus smithsonianus* (centre, inferior) al port de Fuengirola el 19 de febrer de 2019; a dalt a l'esquerra, un adult de gavià fosc *L.fuscus*; a dalt a la dreta, un adult de gavià argentat *Larus michahellis*.



inner web is often broken). However, the right wing P10 had a different pattern with a greater amount of black on the inner web than on the other wing, as well as a relatively small rounded mirror and an uninterrupted black subterminal band between the mirror and the feather tip (Figure 2C). This black band was smaller than the mirror. In both feathers, the mirror did not

extend beyond the rachis and only occupied the inner part of the web. The grey tongue of P10 was relatively short (a little over half the feather length) and not as long as in the Caspian Gull, as Figure 2E shows (seen from below). The tongue had no curved tip and was straight (Figure 2E). According to Adriaens & Mactavish (2004), this sharply ending tongue shape is very common

◀ **Figure 2.** A) American Herring Gull at rest. Note the pale-grey upperparts, pinkish legs, notable white tips to outer primaries, pale-yellow eyes and bill, round head, and stubby body. B) Detail of the head and neck: a pale-yellow coloration predominates on the iris, bill and gape, while the orbital ring is brownish. Only a few light-brown traces are visible on the back of the neck. Compared with the head, the bill is relatively small and similar in size, colour and shape to the Iceland Gull *L. glaucooides*. The bill is pale yellow like the gape. The red gonys spot is only present on the lower mandible, with a faint black mark on its topside. The gonydeal angle is not as marked as in Yellow-legged Gulls. The nail (forward part of the upper mandible) has an oval-shaped patch that is bright-yellow coloured and covers the small area between the nostril and beak tip, like an adult Iceland Gull in winter. C) and D) American Herring Gull in flight showing the wing tip. Note 'thayeri'-like pattern on the left P10 and the following main distinctive features: complete 'W'-shaped black band on P5, 'bayonets' on P7-P8 and a pointed wedge on P6. E) P10 seen from below clearly showing a steeply curved grey tongue at the tip, a key trait for identifying *smithsonianus*. F) Details of upper and underside of wings. P10: the small and almost circular mirror – although reaching the edge of the feather, it is totally confined to the inner web – and the uninterrupted subterminal black band are obvious. P9: visible are the distinctive grey tongue and a lack of mirror. These traits make this gull a good candidate for American Herring Gull from the Great Lakes region or even from the west coast of North America.

A) *Gavià argentat americà en repòs. Les parts superiors són de color gris pàl·lid, amb potes rosades, puntes blanques a les primàries exteriors molt destacades, ulls i bec groc pàl·lid, cap rodó i cos compacte.* B) *Detall del cap i del coll: predomina el color groc pàl·lid a l'iris, bec i comissura bucal i l'anell orbital sembla marronós. Només són visibles algunes traces de color marró clar al coll posterior. En comparació amb el cap, el bec és relativament petit i té una mida, color i forma semblants al de la gavina islandesa *L. glaucooides*. El bec és de color groc pàl·lid igual que la comissura bucal. La taca del gonis vermell només és present a la mandíbula inferior, amb una feble marca negra a la part superior. L'angle gonial no és massa marcat en comparació amb els gavians argentats. L'ungla (part anterior de la mandíbula superior) mostra una taca de forma ovalada amb un color groc brillant que cobreix la petita zona entre la fossa nasal i la punta del bec, similar al de la gavina islandesa adulta a l'hivern.* C) i D) *Gavià argentat americà en vol que mostra la punta de l'ala. Tingueu en compte el patró semblant a "thayeri" a la P10 esquerra i els principals trets distintius: banda negra completa en forma de "W" a P5, "baionetes" a P7-P8 i una falca punxeguda a P6.* E) *La P10 vista des de baix mostra clarament una llengua grisa fortament corbada al final, un tret crític per identificar smithsonianus.* F) *Detalls de la part superior i inferior de les ales. P10: és remarcable el mirall petit i gairebé circular, que arriba a la vora de la ploma, però totalment restringit a la xarxa interior, i la banda negra subterminal ininterrompuda. P9: són visibles una llengua gris característica i la manca de mirall. Aquests trets fan d'aquest gavià un bon candidat per a gavià argentat americà de la regió dels Grans Llacs i, fins i tot, de la costa oest d'Amèrica del Nord.*

in the American Herring Gull but much lesser frequent in the European Herring Gull (being only found in a small percentage of *argentatus* or *argenteus*, in which the mirror is always large in gulls and there is a lot of white on the inner web of P10). These authors point out that the combination of a long ($> 1/2$) grey tongue and very steeply curved shape on P10, as well as a subterminal black band, is a feature found almost exclusively in the American Herring Gull.

Further distinctive key features of the American Herring Gull taxon that reinforced the visual identification included (a) the presence of a complete 'W'-shaped broad black band on P5; (ii) the existence of a distinctive black 'bayonet' on P8 and another shorter 'bayonet' on P7, as well as a pointed wedge on P6 (without, however, being a fully formed 'W' in this case) (Figures 2C-D); and (iii) a fine black marking on the shaft of the outermost covert of P10. While most American Herring Gulls have a 'W'-shaped subterminal band on P5, this trait is very rare in European Herring Gulls (Adriaens & Mactavish 2004). According to these authors, only a very small percentage of *argenteus* have a

black 'W' band on P5; moreover, the nominate *argentatus* lacks this band completely, although this feature is quite variable in eastern Baltic Herring Gulls. Nevertheless, a few adult European Herring Gulls (*argenteus*) may also have black markings on their primary coverts that tend not to be as well defined and neat – or as black – as in many American Herring Gulls (Grant 1986, Lonergan & Mullarney 2004). The combination of these key morphological features was a clear indication that this gull was an American Herring Gull, since it is very unlikely that any individual European Herring Gull would display all these peculiarities at once (Adriaens & Mactavish 2004).

Another significant feature was the lack of a white mirror on P9 and the small size of the mirror on P10, which was confined to the inner web (Figures 2C-D and F). This feature is typical of the American Herring Gull population that winters in the Great Lakes-Niagara Falls region, and of gulls from the west coast of North America (Johnson & Mactavish 2001, Adriaens & Mactavish 2004). According to Olsen & Larsson (2003), there is a clinal var-

iation from the darkest birds on the east coast to paler birds found across northern, central and western North America. This was stated by Dwight (1925) as far back as 1925. The main morphological features concerning this geographical variation in the American Herring Gull are as follows: (a) paler grey upperparts in the west; (b) blacker/less white wing tips in the west than in the east; (c) no mirror on P9 in a high percentage of the west-coast population; and (d) the underside of P10 is mainly black in the western population (Olsen & Larsson 2003). Before this record, the vagrant adult American Herring Gulls observed in Europe have had wing tips that are consistent with those of birds from Newfoundland or the north-east USA (Olsen & Larsson 2003, Adriaens & Mactavish 2004, López-Velasco & Adriaens 2012). Conversely, the Fuengirola bird had (a) extensive black on its outer primaries giving it a blacker wing tip than Newfoundland birds; (b) a small mirror and unbroken subterminal band on P10; (c) no mirror on P9; (d) a grey tongue on the underside of P10 that was not visible at rest (this character is difficult to appreciate under normal conditions); (e) a small, narrow and parallel-edged bill with a delicately curved tip.

Genetic analyses

The results of the mtDNA sequencing using the hypervariable region (HVR) of the control region (CR) and the COI and Cytb genes all assigned unquestionably a Nearctic origin to the gull in this study. The Cytb sequence obtained from the feather (1094 bp) was identical to two GenBank sequences, one from *L. smithsonianus* and one from *L. californicus*, which is known to hybridize with *smithsonianus* (Chase 1984). Details of the Cytb sequence are included in the Supplementary Material. The nearest *argentatus* had four different bases, which indicates how close taxonomically all these gulls are. Figure 3 shows the phylogenetic reconstruction from the concatenated CR/Cytb sequences and the clear proximity of the Fuengirola gull to *smithsonianus*. Bayesian reconstructions from the partial sequences of the Cytb (812 bp) and COI (615bp) mtDNA genes are also provided in the Supplementary Material (Figure S1).

In the COI data the alignment consisted of 615-bp sequences of 30 taxa that included

the Fuengirola gull plus 29 other homologous sequences from GenBank. The Bayesian reconstruction clearly clustered the Fuengirola gull with all the American species, along with others from Japan and Korea, and was very close to *smithsonianus* (Figure S1). The Cytb alignment consisted of 812-bp homologous sequences including the sample plus 42 taxa from GenBank. The Bayesian reconstruction in this case is more informative than the COI-based one and again supports significantly the clustering of the Fuengirola gull with *L. smithsonianus* (Figure S1). Furthermore, this fragment suggests significantly that the Fuengirola gull was more closely related to samples from the Great Lakes (Figure S1) and from Prince Edward Island (Canada). To summarise, both markers and, particularly, the Cytb point to *smithsonianus*.

Discussion

The first modern record of an American Herring Gull in Spain (only preceded by a bird ringed in 1936 as immature and observed at sea 480 km NW off Cape Finisterre in 1937) was in Gijón (Asturias) on January 1991 (de Juana 1995). This was followed by three more gulls in Getxo (Biscay) in January 2006 (Díes *et al.* 2008), Cariño (A Coruña) in March 2007 (Díes *et al.* 2009) and Colmenar Viejo (Madrid) in February 2010 (Gutiérrez *et al.* 2012). Two of these locations are on the Atlantic coast of northern Spain (Getxo and Cariño), although the third is in the centre of the country (Madrid). Two were in first-winter plumage (Atlantic coast) and one in second-winter plumage (Madrid), as is the case of most records from Europe. By contrast, the American Herring Gull observed in Fuengirola was the first-ever observation of an adult, which wanders less frequently. Vagrancy is relatively common in first-year birds both naturally and when birds are ship-assisted. However, the birds from Biscay and A Coruña returned to the same localities for several winters and were named 'Barrilete' and 'Ondarru' by local ornithologists and eventually became the first adult American Herring Gulls observed in Spain. The identification of these three individuals was based wholly on morphological features.

By contrast, the Fuengirola record was also based on molecular evidence. Large Nearctic

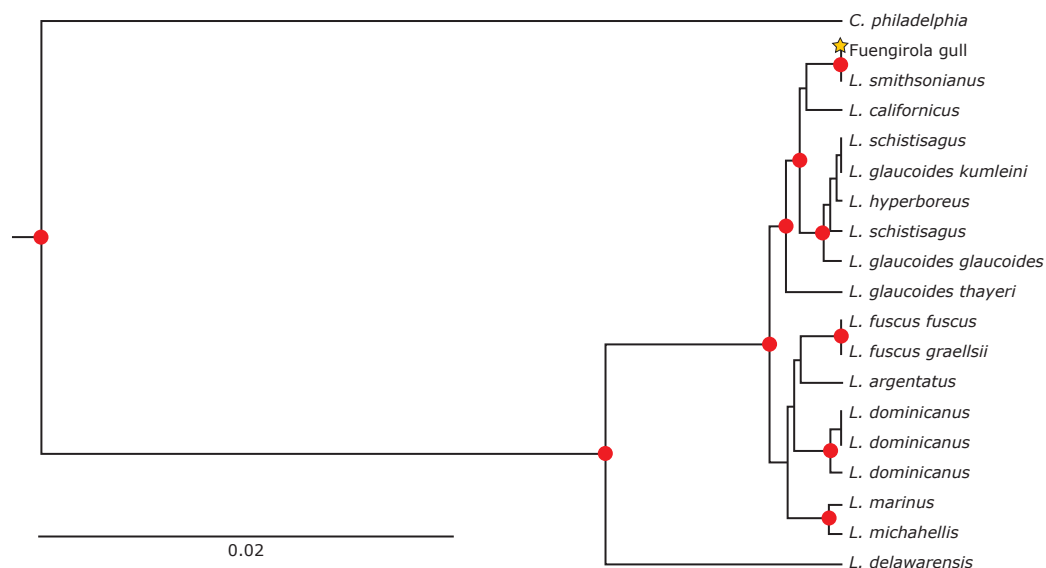


Figure 3. Tree from concatenated CR/Cytb sequences obtained using the feather sample from the Fuengirola gull. Support values corresponding to bayesian posterior probabilities >0.98 highlighted. *Arbre de seqüències concatenades de CR/Cytb obtingudes de la mostra de plomes del gavià de Fuengirola. Es destaquen els valors de suport corresponents a probabilitats bayesianes posteriors >0,98.*

gulls (*smithsonianus*, *schistisagus*, 'American' *hyperboreus*, *thayeri*, *californicus*, *vegae* and *mongolicus*) hybridise. Nevertheless, the mtDNA analysis identified the samples to species level and clearly showed that this gull was of Nearctic/Pacific origin, precluding the possibility of any European taxon. The combination of these genetic analyses and the plumage features is strong evidence for the correct identification of the American Herring Gull observed in Fuengirola as *L. smithsonianus*. Thus, this finding extends the range of vagrant American Herring Gulls as far as the Western Mediterranean, a previously undocumented occurrence.

Additionally, on the basis of the patterning on the wing tip, there is a high probability that this bird originated from the population wintering in the Great Lakes region or even on the West coast (Johnson & Mactavish 2001, Olsen & Larsson 2003). This was the first time that a gull with this combination of characters had been observed in Europe. Thus, although in this case the Cytb sequence is unable to confirm a western or central origin for this gull, its DNA is quite similar to sequences available in GenBank for gulls from these regions (Figure S1). The 'thayeri' pattern on the left wing, very pale upperparts and bill and body structure could

potentially indicate genetic influence from the *thayeri/glaucooides* taxa that breed in the Canadian Arctic and winter on the Pacific Coast and in the Great Lakes region of North America; even so, to date no such hybridisation has ever been recorded (Olsen & Larsson 2003, Howell & Dunn 2007, Olsen 2018) and so this possibility should be excluded.

From a biogeographic perspective, is important to keep track of these records of vagrant gulls as they contribute to and improve our knowledge of species distribution, above all in gulls, which may be confused with similar species when they are outside their normal ranges.

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Xabier Saralegui, J. A. Sarrión, Xabier Varela, Nacho Vega and Montserrat Vilosa.

Resum

Anàlisis morfològiques i genètiques revelen la presència del gavià argentat americà *Larus smithsonianus* al Mediterrani

Donem a conèixer el primer registre d'un gavià argentat americà *Larus smithsonianus* en un àrea costanera del Mediterrani durant febrer-març de 2019 a Fuengirola, Màlaga, sud d'Espanya. La gavina era un exemplar adult i la identificació es va confirmar mitjançant la combinació d'anàlisis moleculars d'ADN mitocondrial i de caràcters morfològics. L'observació destaca per ser possiblement la primera gavina amb fenotip "Niagara" observada a Europa, indicant un origen vinculat a l'àrea central i, fins i tot, a la costa oest de l'Amèrica del Nord. Es tracta del registre més al sud d'un exemplar adult per a aquesta espècie a la regió Paleàrtica occidental, i seria l'onzè per a la península Ibèrica des de 1991.

Resumen

Análisis morfológicos y genéticos revelan la presencia de la gaviota argénteo americana *Larus smithsonianus* en el Mediterráneo

Damos a conocer el primer registro de una gaviota argénteo americana *Larus smithsonianus* en un área costera del Mediterráneo durante febrero-marzo de 2019 en Fuengirola, Málaga, sur de España. La gaviota fue un ave adulta y la identificación se confirmó mediante la combinación de análisis moleculares del ADN mitocondrial y de caracteres morfológicos. La observación destaca por ser posiblemente la primera gaviota con fenotipo "Niagara" observada en Europa, indicando un origen vinculado al área central e incluso a la costa oeste de Norteamérica. Se trata del registro más al sur de un ave adulta para la especie en la región Paleártica occidental, y sería el onceavo para la Península Ibérica desde 1991.

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Supplementary material / Material suplementari

Supplementary material may be found in the online version of this article / Es pot trobar material suplementari de la versió en línia d'aquest article.

Figure S1. Phylogenetic relationships of the Fuengirola gull based on a) 615-bp COI fragment and b) 812-bp Cytb mtDNA fragments obtained using a Bayesian criteria. The Bayesian posterior probabilities partitioned the data by character. The Fuengirola gull is shown in red. (See text for details of the analyses). *Relacions filogenètiques del gavià de Fuengirola basades en a) un fragment COI de 615 pb; b) fragments de mtDNA de Cytb de 812 pb obtinguts a partir d'un criteri bayesià. Les probabilitats posteriors bayesianes van obtenir la partició de les dades per caràcters. El gavià de Fuengirola es mostra en vermell (vegeu el text per obtenir informació detallada sobre l'anàlisi).*

Supplementary material / Material suplementari

DNA extraction and sequencing

In Aberdeen, the genomic DNA was isolated from feather and faeces using a QIAamp DNA Micro Kit (Qiagen, UK) according to the manufacturer's instructions, with the addition of dithiothreitol to 0.1 M concentration in the proteinase K digestion mix and elution in 80 μ L of Qiagen buffer AE. DNA fragments were amplified via polymerase chain reaction (PCR) using primers and protocols as described in Helbig *et al.* (1995) for Cytb. Amplification products were gel-purified using the QIAGEN Gel Extraction Kit according to the manufacturer's instructions, with a final elution in 30 μ L Buffer EB. Sanger sequencing was performed by Source BioScience (Nottingham, UK).

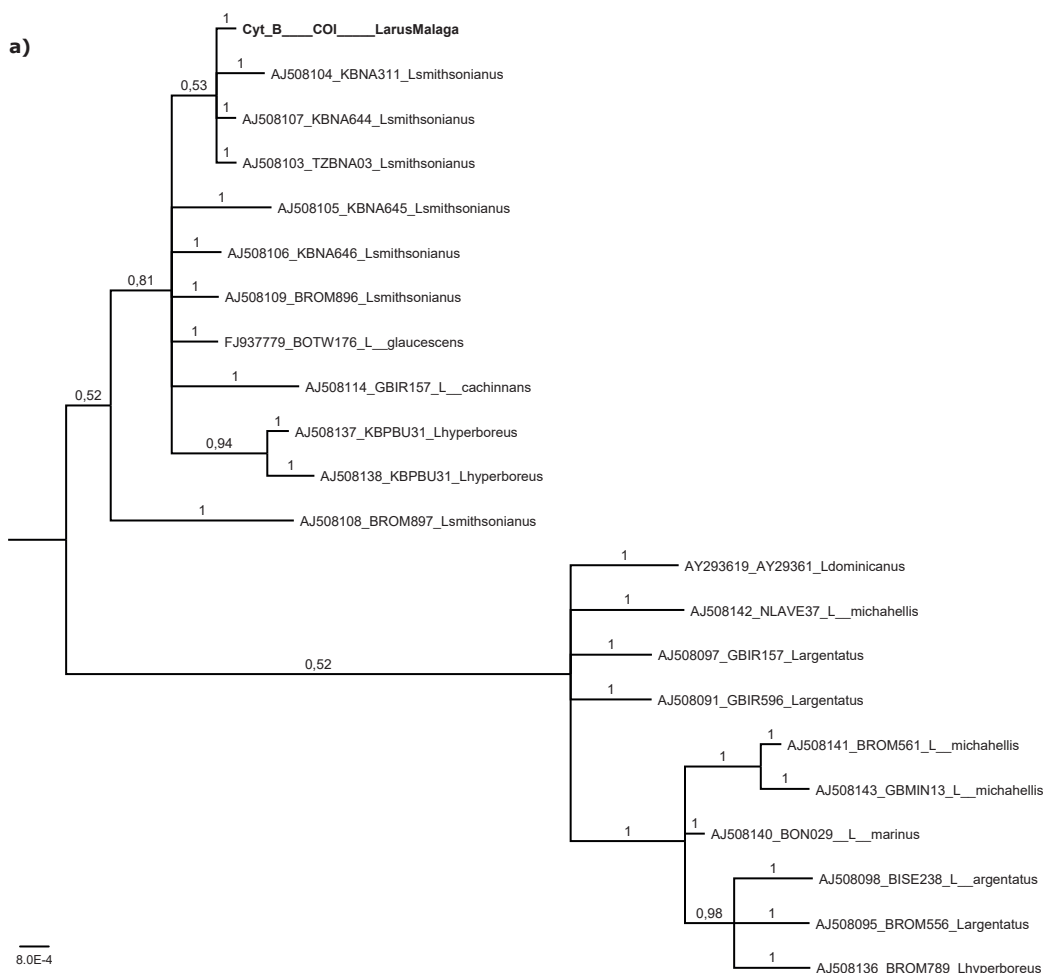


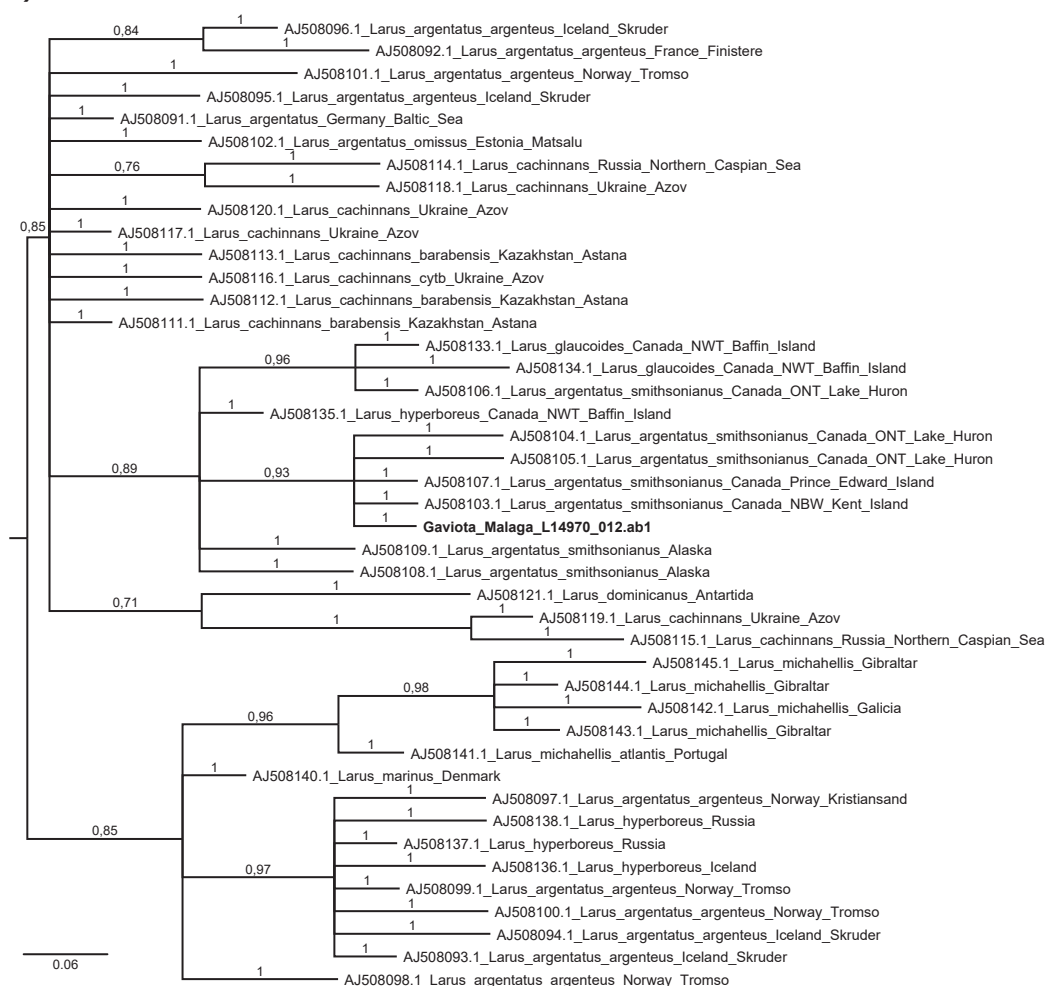
Figure S1. Phylogenetic relationships of the Fuengirola gull based on a) 615-bp COI fragment and b) 812-bp Cytb mtDNA fragments obtained using a Bayesian criteria. The Bayesian posterior probabilities partitioned the data by character. The Fuengirola gull is shown in red. (See text for details of the analyses). *Relacions filogenètiques del gavià de Fuengirola basades en a) un fragment COI de 615 pb; b) fragments de mtDNA de Cytb de 812 pb obtinguts a partir d'un criteri bayesià. Les probabilitats posteriors bayesianes van obtenir la partició de les dades per caràcters. El gavià de Fuengirola es mostra en vermell (vegeu el text per obtenir informació detallada sobre l'anàlisi).*

Sequences were checked visually and were aligned in CLC Sequence Viewer (<http://www.clcbio.com/products/clc-sequence-viewer/>) using default parameters with representatives of other *Larus* species available in GenBank (<http://www.ncbi.nlm.nih.gov/>). A maximum-likelihood tree was generated in IQ-Tree with default settings.

In Spain, total DNA was extracted starting from 0.01 to 0.05 g of the faecal sample using a modified guanidinium thiocyanate (GuSCN) method described by Rohland & Hofreiter (2007) following Pastor-Beviá *et al.* (2014) and Ibáñez *et al.* (2016). The mitochondrial fragments of avian DNA were amplified using a different combination of primer sets (1) around 600 bp of the COI using primer sets COIPrey (FW/RW) (Pastor-Beviá *et al.* 2014), and (2) around 800 bp of the Cytb in both directions using the primer sets CytbSPrey (FW/RW) and CytbLPrey (FW/RW). Amplification products were sequenced in an ABI 3100 automated sequencer (PE Biosystems, Warrington, UK) following the manufacturer's protocols. The obtained sequences were checked visually with Sequencher v. 4.9 (Gene Codes Corp, MI, USA).

The Fuengirola gull sequence was aligned with multiple Cytb and COI sequences from other large white-headed gulls from the Palearctic and Nearctic that we selected from a search of the GenBank and BoldSystem databases (<http://www.boldsystems.org/>) for each marker alone and the concatenated sequences. Species identification was based on a >98% of similitude threshold.

b)



Bayesian reconstructions for each COI and Cytb fragment and for the concatenated alignment were obtained with a Bayesian criteria using MrBAYES v. 3.1.1. (Huelsenbeck & Ronquist 2001) portioning the data set by character. The Bayesian topologies were obtained after five simultaneous Markov chains were run for 2 million generations. Trees were sampled every 100 generations with a resulting burn-in of 25% of the sampled trees.

Fuengirola gull complete Cytb sequence

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CTACTAGGCATTTGCCTACTAACACAAATCCTAACAGGACTCCTGCTAGC
TATACATTACACCGCAGACACAACCCTAGCCTTCTCATCCGTCGCCACA
CATGTTCGAAACGTACAATATGGCTGACTAATCCGAAACCTCCACGCAA
ACGGAGCGTCATTCTTCTTTATTTGTATTTACCTACACATCGGACGAGGATTC
TACTATGGCTCATACTCTATAAAGAAACCTGAAATACAGGAGTCATTCTCCTCT
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CACCTAACCTTCCTTCACGAATCAGGATCAAACAATCCACTGGGTATTCAATCAA
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CAGAAAACCTTTACTCCAGCAAACCCACTCGTTACACCCCCACACATCAAACCCGA
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