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Title: Impacts of biological globalization in the Mediterranean: unveiling the deep history of human-mediated gamebird dispersal.

Short title: Impacts of biological globalization in the Mediterranean

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SIGNIFICANCE: Human-mediated species dispersal stretching back at least 10,000 years has left an indelible stamp on present day biodiversity. A major contributing factor to this process was the trade in a wide range of exotic species that was fueled by elite demand. The black francolin, now extinct in the western Mediterranean, but once a courtly gamebird prized for its flavor, curative and aphrodisiac qualities by European aristocracy, was one of these species. Using historical sources and DNA analysis of modern and archival specimens, we show that this bird was not native to the western Mediterranean, and document its introduction to Cyprus and westward through the Mediterranean Basin via several trade routes that reached as far east as South Asia.

ABSTRACT. Humans have a long history of moving wildlife that over time has resulted in unprecedented biotic homogenization. It is, as a result, often unclear whether certain taxa are native to a region or naturalized, and how the history of human involvement in species dispersal has shaped present-day biodiversity. Although currently an eastern Palaearctic galliform, the black francolin (*Francolinus francolinus*) was known to occur in the western Mediterranean from at least the time of Pliny the Elder if not earlier. During Medieval times and the Renaissance, it was a courtly gamebird prized for its flavor, curative and even aphrodisiac qualities. There is uncertainty, however, whether this important gamebird was native or introduced to the region and, if the latter is the case, what the source of introduction into the western Mediterranean was. Here we combine historical documentation with a DNA investigation of modern birds and archival (13th - 20th century) specimens from across the species' current and historically documented range. Our study proves the black francolin was non-native to the western Mediterranean, and we document its introduction from the east via several trade routes, some reaching as far as South Asia. This provides insight into the reach and scope of long-distance trade routes that serviced the demand of European aristocracy for exotic species as symbols of wealth and prestige and helps to demonstrate the lasting impact of human-mediated long distance species dispersal on current day biodiversity.

INTRODUCTION

Human-mediated species translocations have played a central role in shaping global biodiversity for thousands of years (1). The dispersal of early agricultural economies out of the centers of initial domestication more than 10,000 years ago marks an acceleration of human directed species range expansions involving both domesticates and a wide range of non-domesticated species (2, 3). The maritime and overland trade routes of the third millennium BC that linked major urban centers across South and Central Asia, Mesopotamia, the Arabian Peninsula, and North Africa expanded the geographic distribution and diversity of species through long distance translocations (4). The range and impact of this process continued to increase as transportation technology improved and as demand for both staple and rare, exotic species from faraway places grew among ruling elites and rising mercantile classes across the increasingly vast territory connected in these exchanges. The post AD 1000 period in particular saw a surge in species translocations as emerging nation states in Medieval and Renaissance Europe received a staggering diversity of plants and animals through trade routes that linked an expansionist Islamic world with major empires in central Asia and China (1, 5).

This process of biological globalization resulted in large-scale reshuffling of both wildlife and domesticates that has had an especially profound and lasting impact on native biotas in the Mediterranean Basin. Species translocations have led to substantial if not complete replacement of insular endemics (6). At the same time, human-mediated species movement and landscape management have helped preserve high biodiversity of present-day anthropogenic, yet threatened, Mediterranean environments (7). The impressive pace and extent of present-day wildlife relocations raises concerns about “biotic homogenization”, the loss of biological distinctiveness in regions following replacement of native biotas by locally expanding non-natives (8). Achieving a comprehensive understanding of the antiquity and impact of humans on Mediterranean biodiversity promises significant insight into ongoing conservation issues, and sheds new light on the role of long distance trade and exchange in shaping the cultural identities and national destinies of people across the Mediterranean Basin.

With their colorful plumage, small size, and relative ease of transport and management, birds are likely candidates for long-distance exchange and were often the animal of choice in European menageries (9, 10). The peacock (*Pavo cristatus*), for example, is thought to have been imported from Asia to Greece during the time of Alexander the Great, and perhaps even earlier (11). In his *De arte venandi cum avibus*, Frederick II referred to the importation of the Guinea fowl (*Numida meleagris*) from the Levant to Sicily (12). Chronicles of European adventurers in Asia such as Marco Polo (13) often contained references to gamebirds. Since the first centuries BCE, travelers along the Silk Road are known to have carried and bred chukar partridges (*Alectoris chukar*) as a source of food on the way to Europe (14).

Another species of gamebird that may well have been included in these exchanges is the black francolin (*Francolinus francolinus*, Phasianidae). No longer found in the western Mediterranean, the black francolin is known from textual and iconographic sources as a gamebird species that figured prominently in courtly life in Medieval and Renaissance Europe (5, 15). Here we combine historical documentation with the genetic study of modern, archaeozoological, and archival collections of these birds to assess whether the black francolin represents an example of the extirpation of a native or an introduced species in the western Mediterranean, thus exploring what this species can tell us about the nature of human-mediated dispersals in the region.

The black francolin in the Mediterranean

The black francolin is a Palaearctic medium-sized gamebird (Fig. S1) that is presently distributed, with some notable interruptions, from the Near East and Central Asia to Bangladesh (Fig. 1: A). This sedentary, non-migratory species inhabits a variety of lowland open habitats, showing marked preference for cultivated and wetland ecotones bordering marshes, riversides and lake edges with scrub and thickets (16). A recent molecular study (17) revealed three strongly differentiated mitochondrial DNA haplogroups including a pair of morphological subspecies each (*F. f. francolinus-F. f. arabistanicus*, *F. f. bogdanovi-F. f. henrici*, and *F. f. asiae-F. f. melanonotus*). The black francolin was once, however, found throughout the western Mediterranean, although until now it was not known whether the species was native or introduced.

The black francolin is thought to be the so-called *Attagen*, which appears for the first time in a comedy (*Ornithes*, "The Birds") written by the Greek dramatist Aristophanes (414 BC) and performed for the Festival of Dionysus (18). The *Attagen* is later featured in a poem (*Xenia*, "Gifts") by the Roman epigrammatist Martial (*ca*. 85 BC) where it is referred to as a particularly tasty bird included among the presents sent home with party guests at the festival of the *Saturnalia*. Later still, it is mentioned by the Roman lyric poet Horace (30 BC) in his iambic poetry (*Epodes*, "The Epodes") as follows: *Non Afra avis descendat in ventrem meum, non attagen Ionicus iucundior quam lecta de pinguissimis olive ramis arborum* (Not African fowls, nor Ionian *Attagen* could pass my lips more happily than the fruit collected from the most heavily loaded branches of the olive). In his *Naturalis Historia* (77 AD) Pliny the Elder reported: *Attagen maxime Ionius celeber et vocalis alias, captus vero obmutescens quondam extimatus inter raras aves, iam et in Gallia Hispanique* (the most reputed *Attagen* is that from Ionia; it usually sings but is silent in captivity; once considered a rare bird, but now it is also found in Gaul and Spain) (19).

No additional textual documentation of the black francolin in the Mediterranean exists until the Medieval and Renaissance periods, when the bird figures prominently among a range of courtly game species highly prized by European nobility as a sign of great power and prestige (20), with severe bans that limited hunting of the black francolin to a privileged few (15).The earliest secure documentation for the presence of the black francolin in the western Mediterranean is a letter in 1368 sent by the Spanish king Peter IV of Aragon from Sicily to the governor of Mallorca, which specifically mentions the black francolin as one of a number gamebirds introduced to the island (21). Not only was the meat of the black francolin held in high regard because of its delicate flavor, it was also thought to possess curative (15) or even aphrodisiac properties (22, 23). Interestingly, the related African francolins appear among the species exploited and traded in African traditional medicine for much the same reasons (24). According to the Islamic medical tradition, the consumption of black francolin meat was recommended to pilgrims travelling to Mecca due its digestibility (25).

During the Renaissance, the possession and display of exotic birds was greatly in vogue among the aristocracy (26, 27), embodying the allure of diversity and novelty that is a common thread in the history of the importation of exotic species (9, 28).The black francolin was included among the highly valued gamebirds of the era. In fact, its introduction into central Italy from Sicily in the second half of the 15th century is attributed to Lorenzo the Magnificent, who imported some individuals to keep as ornamental birds in his model farm of Poggio a Caiano near Florence (15, 29). The role of this gamebird as a symbol of elite status and prestige during the Renaissance is captured in the painting "The Hunters Game" by Justus Sustermans, who served as court painter to the Medici family in 17th century Florence (Fig. S2). In it a black francolin is featured as one of the animals taken in a hunt by a number of clearly high-born hunters.

Up to the mid-19th century the range of the black francolin in the Mediterranean extended from Greece across Sicily and southern Italy west to Spain (Fig. 1: B) (30). Within a few decades in the late 1800s, however, the combined effects of overhunting and land reclamation resulted in the extirpation of all populations of this gamebird from the western Mediterranean (21, 31). The scanty and fragmentary information available on this species coupled with the lack of archaeozoological specimens of the bird in this region, as well as the misleading use of multiple common names, has prevented ornithologists from determining whether the western Mediterranean's first black francolins (i.e. those known from historical records in classical times) were endemic to the region or part of the wide array of non-native species introduced during the long history of human-mediated species dispersals in the region.

For a number of different taxa, the study of ancient or historical DNA extracted from archaeozoological remains and older archival specimens has proven key in addressing questions of autochthony *versus* allochthony (32, 33) and in tracing human-mediated dispersal (34, 35). In this study we investigated the mitochondrial DNA of modern and archival specimens (13th - 20th century) collected across the black francolin's current and historical range to elucidate the enigmatic origin of the now extinct *F. francolinus* in the western Mediterranean (Fig.1, Table S1). The sample of modern birds includes 205 specimens collected between 2007 and 2013. In addition, we also analyzed 76 samples from archival specimens housed in museums in the US and Europe, including 17 specimens from regions where the black francolin is currently extinct. One of these was an archaeozoological specimen identified as belonging to a black francolin recovered in excavations of 13th century Arab-Normal castle of Calathamet in Sicily (36). This is the only known such specimen identified as a black francolin in the western Mediterranean. Here we present evidence that argues for the non-native status of the black francolin in the western Mediterranean. We document multiple introductions of the species, including introductions from the far eastern reaches of its current distribution, shedding new light on the impact of medieval wildlife reshuffling on the distribution of courtly gamebirds and on the role of human-mediated dispersals in shaping biodiversity of the western Mediterranean.

RESULTS

The two mtDNA sequences obtained from the archaeological bone sample retrieved at Calathamet were 100% identical and were assigned to a Sicilian rock partridge (*Alectoris graeca whitakeri*); hence, this haplotype (H73: LK871855 GenBank code: Table S2) was eliminated from the dataset. The re-identification of this specimen leaves the 14th century letter from the king of Aragon (21) that mentions the bird as having been introduced to the island as the earliest secure record of the presence of the black francolin on Sicily. The alignment of the 185-bp long CR fragment of remaining 281 sequences defined a set of 186 characters, indels included. There were 49 variables sites: among these, 31 were parsimony informative. Seventy-two haplotypes were found (H1-H72: LK871783-LK871854 GenBank accession codes; Table S2). Bayesian phylogeny (Fig. 2) clustered all haplotypes hold by *francolinus-arabistanicus* (H1-H23) and *bogdanovi-henrici* (H46-H72) black francolins into highly reliable clades (posterior probability = 0.93 and 0.99, respectively: a 0.90 credible set contains 86,401 trees). All haplotypes hold by *asiae-melanonotus* (H24-H45) birds were basal to mentioned groups. Specifically, (i) the *francolinus*-*arabistanicus* clade harbored the samples from the westernmost part of the species' range including archival specimens from Sicily and Tuscany holding the most common haplotypes in Cyprus (H1, n = 1; H20, n = 6) (Table S1). On the other hand, (ii) the *bogdanovi-henrici* clade hosted specimens from central Asia, India, and Nepal, as well as 19th century black francolins from Sicily (H70 and H71, n = 2) and Spain (H61, n = 1). The haplotype held by this latter was shared with Indian and Pakistani conspecifics. Finally, (iii) basal *asiae-melanonotus* included birds from Pakistan, India, Nepal, and Bangladesh plus archival black francolins from Sicily holding haplotypes either private (H33, n = 1) or common to Indian and Pakistani individuals (H26, n = 1; H30, n = 1). Overall, Sicilian specimens (n = 11) showed a high level of diversity (h ± SD = 0.82 ± 0.12, with two private haplotypes clustering in the *bogdanovi-henrici* group). The short length of the CR gene fragment sequenced notwithstanding, Bayesian phylogeny displayed overt correspondence to the three clusters provided by the network (Fig. S4), each containing the same haplotypes found in the analogous clades of the tree. Furthermore, Bayesian phylogenetic reconstruction perfectly matched with the black francolin adaptive radiation inferred by Forcina et al. (17) sequencing the entire CR gene but relying on a smaller sample size.

DISCUSSION

Francolinus f. francolinus - F. f.arabistanicus

The most common subspecies detected among the archival specimens studied here belong to the *francolinus-arabistanicus* group, found in the western-most extent of the modern range of the black francolin. Within this group, archival specimens from Cyprus (n = 12) belong predominately to two haplotypes (H1 = 8, H20 = 3), which also predominate among the 59 modern samples from Cyprus included in our study (H1 = 30, H20 = 27). The H1 and H20 haplotypes are mostly absent in neighboring areas on the mainland. All nine of the modern and archival specimens from Israel, for example, belong to the H13 haplotype of this subspecies. The only other H20 specimen from the eastern Mediterranean is an archival specimen from Izmir on the western coast of Turkey.

Reports on the avifauna of paleontological and archaeological sites in Cyprus are rare, but there are no black francolin remains recorded in the large avifaunal assemblage from the Akrotiri rock shelter, which both pre-dates human arrival on Cyprus and captures some of the initial visits to the island by hunters from the mainland (37). Nor is the species reported in the assemblages of the initial pre-ceramic colonists to the island responsible for the importation of a number of domestic and wild game animals (2). On the other hand, the black francolin is relatively well represented among the avifauna assemblages at Epipaleolithic and Neolithic sites in Iraq (38), Israel (39), and Syria (40). The first osteological record attributed to the species on Cyprus dates back to the Middle Bronze Age (41). This gamebird, then, was most likely introduced to Cyprus from the mainland sometime after the initial human colonization in the pre-ceramic Neolithic.

There is ample documentation that sea-faring colonists imported a wide range of economically relevant mainland fauna to Cyprus beginning as early as 11,000 years ago

(42). Prior to human colonization, the endemic fauna of Cyprus was impoverished, consisting of pygmy hippos and elephants (extirpated soon after the initial human visitation of the island, if not before), a species of genet, and a couple of endemic bat and rodent species (43). Early imports to the island included not only domestic or at least managed livestock species (pigs, goats, cattle, and sheep) but also a variety of game species, most notably Mesopotamian fallow deer (*Dama dama mesopotamica*) and the red fox (*Vulpes vulpes*) (2). The consensus opinion is that the original source of introduced livestock and game species on Cyprus in ancient times was the northern Levant or coastal Anatolia (41, 44), and an active exchange of people and resources between Cyprus and Asia Minor from the Late Neolithic onward is well documented (45). These historical connections make the presence of the H20 haplotype in Turkey especially interesting, and suggest Asia Minor as the possible source population for at least some of the black francolin populations on Cyprus. A parallel to the now extinct population of the black francolin in western Turkey might well be the local European fallow deer (*Dama dama dama*) that was once widespread in the region, but is now seriously threatened. Closely related mitochondrial lineages of this Anatolian population of fallow deer, however, persist among introduced conspecifics on the near-by island of Rhodes (46).

Moving westward, the H20 haplotype of the *francolinus-arabistanicus* subspecies of black francolin is the most common group among the archival specimens from Sicily (H20 = 5), with an additional Sicilian specimen assigned to this subspecies belonging to the H1 haplotype. This affiliation with the haplotypes found on Cyprus (and virtually nowhere else) strongly points to Cyprus, or possibly the western coast of Turkey, as a source of the black francolins on Sicily. Other studies based on historical documentation have proposed that Crusaders were responsible for the importation of the black francolin from Crusader controlled territories on Cyprus, mainland Palestine, and Asia Minor to Sicily (15, 47). The close affiliation of the mitochondrial lineages of the Sicilian specimens with those from Cyprus lends support for this thesis. The single archival specimen from mainland Italy (Tuscany) also belongs to this ubiquitous H20 haplotype, harkening to the above mentioned historical documentation of the importation of black francolins from Sicily to Tuscany during the time of Lorenzo the Magnificent (15). Once again, while it cannot be certain that this was the context of the initial import of these birds to mainland Italy, our analysis does point to Sicily as a source of birds originally imported to the western Mediterranean from Cyprus and the eastern Mediterranean.

Francolinus f. bogdanovi - F. f. henrici* and *F.f. asiae - F. f. melanonotus

While the affiliation of archival specimens from Sicily to the *francolinus-arabistanicus* group was not unexpected, the identification of other specimens from the western Mediterranean to subspecies currently found in South Asia represents the most interesting result of this study. Two of the Sicilian archival specimens belong to the subspecies *bogdanovi-henrici* (H70 = 1, H71 = 1) found today in southeastern Iran, Afghanistan, and Pakistan, while three more specimens belong to the subspecies group *asiae-melanonotus* (H26, H30, and H33) that today can be found in a region that stretches from Northeastern India, across Nepal, to far eastern northern India (Fig. 1A, Fig. S3).Thus, nearly half (5 out of 11) of the Sicilian black francolin specimens sampled here were derived from populations ranging across a broad area from western to eastern South Asia. Moreover, the only Spanish specimen obtainable from archival collections is associated with the western-most South Asian subspecies *bogdanovi-henrici* (H61). Thus, although the number of archival specimens from the western Mediterranean is unavoidably small, the prominence of haplotypes ascribable to these far removed subspecies speaks to the importance of these distant locales as major sources for the introduction of the black francolin into the region.

Given the far-flung commercial routes that connected the Mediterranean Basin with Central, South, and eventually East Asia from the Bronze Age onward (Fig. S3), it seems quite plausible that black francolins native to South Asia were included among the diverse plant and animal taxa that traveled along these trade routes (1).The Aragonese naturalist Diego de Funes y Mendoza (17th century) in his translation of the *Historia Animalium* by Aristotle (*ca*. 340 BC), refers to the black francolin as an *Asian bird introduced to France and Spain* (21). Although it is difficult to know what exactly he meant by *Asia*, which in the 17th century Spanish literature of exploration was as broadly defined as it is today (48), in view of our data the source of these birds may just as well likely be South Asia as the Near East. From the 14th century onwards, Lisbon acted as an emporium for a remarkably diverse range of goods from all over the then-known world (49). Portuguese kings sent exotic or rare animals obtained through this trade, including birds, with embassies as gifts to other European rulers - a practice that was common in most parts of the world until well into the 19th century. It is likely that the highly sought after black francolin played a role in this custom. Portuguese merchants, then, may well be responsible for bringing at least some black francolin stocks to Europe from these distant locales (Fig. S3).

The shared western South Asian origin of the Spanish sample with Sicilian black francolin specimens belonging to the *bogdanovi-henrici* subspecies group points to the island as an important node in the exchange routes that brought these birds to Spain, as well, as suggested earlier, to mainland Italy. Sicily was conquered and incorporated into the Catalan-Aragonese Confederation in 1282, which held dominion over large portions of the Mediterranean Basin, from parts of France and Spain to parts of Greece (21). The close correspondence between the former distribution of the black francolin in the Mediterranean and the territory controlled by the Catalan-Aragonese Confederation during the Renaissance suggests that this maritime empire may have played a role not only in the import of this exotic species from South Asia, but in its dissemination throughout the Mediterranean Basin (22, 50-52).

Regardless of the time frame, at the very least our data suggest that Sicily, located strategically as a convenient way station for cross-Mediterranean trade networks from early prehistory, played a major role in the species' stepwise colonization of the western Mediterranean. While the 71 modern and archival specimens in our study from Cyprus belong to four closely related haplotypes within the *francolinus-arabistanicus* subspecies group (with over 95% of the specimens sampled belonging two closely related haplotypes), the Sicilian archival specimens showed a very high degree of haplotype diversity, with seven different haplotypes representing all three subspecies haplogroups. Sicily, then, would seem to have been a central point that drew in black francolins from Cyprus, as well as those coming from much more distant locations in South Asia through trade routes, like those controlled by the Portuguese, that may have by-passed Cyprus

(Fig. S3), and from which these exotic and highly prized gamebirds were distributed throughout the western Mediterranean.

Finally, it is worth noting that the commercial black francolin stocks introduced either worldwide during the 20th century (e.g. to US mainland and Pacific islands, see ref. 16) or very recently (e.g. as pets to Cyprus, see ref. 53), originate from the Indian sub-continent (*F. f. asiae* subspecies). This practice closely resembles recent human-mediated introductions of gamebirds such as the chukar partridge (*Alectoris chukar*) and the Japanese quail (*Coturnix japonica*) from the East to the Mediterranean (54, 55), and shows that early human-mitigated dispersal of gamebirds continues today in an even more amplified form. We suggest, then, that reconstruction of the role played by historical trade routes in the dispersal of species like the black francolin provides an important deep time perspective that will be useful in the present day management of the avian biodiversity. As such, this study adds to the growing body of cross-disciplinary research that brings together diverse data sets from the humanities and the sciences to illuminate the history of human-mediated long distance movement of species, a process of biological globalization which continues to shape our world today.

MATERIALS AND METHODS

Biological sampling: modern birds

We sampled 205 modern black francolin between 2007 and 2013 (Fig. 1: A, Table S1). Of these, 200 samples were featured in Forcina *et al.* to address the molecular evolution of the genus *Francolinus* as a whole (17), while five were newly collected. Samples were 96% ethanol preserved and stored at - 40 °C after delivery.

Biological sampling: archival specimens

Seventy-six tissue samples (slivers of toe pads, feathers) from *F. francolinus* archival specimens held in US and European natural history collections and collected over a period from 1838 to 1954 were loaned for this study (Fig. 1: A, B and Table S1). Archival specimens from regions where the black francolin is currently extinct include eleven from Sicily and one from Tuscany (Table S1). An archival Spanish specimen, the only one existing after those recorded by Lord Lilford (31) were lost during the Valencia University fire of 1932 (21), was also included among the selected specimens. Efforts at locating archaeological specimens of black francolins from the western Mediterranean were largely unsuccessful. This is not surprising given (i) the rarity of the species in antiquity, (ii) the difficulty in identifying the bones of closely related phasianidae taxa, and (iii) the incomplete archaeozoological record from later time periods in the region (9). The only archaeozoological specimen included in the sample was a coracoid found among the faunal remains excavated at the Arab-Norman castle of Calathamet (north western Sicily). Identified as a black francolin based on its morphology (36) it is believed to date to the 13th century.

Modern bird DNA extraction

All modern DNA extractions were conducted at the Department of Biology of the University of Pisa (Zoology and Anthropology Unit - Zoology building). The DNeasy

Blood and Tissue Kit (Qiagen) and the Puregene Core Kit-A (Qiagen) were used to extract DNA from feathers and liver samples, respectively, and following the manufacturer's instructions. A 2 mm-long fragment was cut from the proximal tip of each feather, while roughly 20 mg of tissue was removed from each piece of liver. The reliability of each DNA extraction was checked through negative controls (no tissue added). DNA concentration and purity was assessed with an Eppendorf BioPhotometer (AG Eppendorf).

Archival specimen DNA extraction

DNA extractions of archival specimens were carried out in a dedicated room free of any *Francolinus* DNA at the Department of Biology of the University of Pisa (Zoology and Anthropology Unit - Anthropology building). The selection of physically isolated venues to process archival and modern samples aimed at preventing contamination. A small amount of starting material (≤ 5 mg) was removed from toe pad or feather fragments and minced employing a sterile disposable razor blade (BBraun, Aesculap Division). DNA was isolated using the QIAamp DNA micro kit (Qiagen) in compliance with the manufacturer's instructions, modified as follows when dealing with very hard tissues: (i) incubation in a shaking water bath up to 48 h; (ii) use of 3 µl of dithiothreitol (Fluka, 100 mg/ml); (iii) twofold addition of proteinase K (Sigma Aldrich, 20 mg/ml); (iv) repeated freezing and thawing of the supernatant as suggested in Pergams and Lacy (56). A small amount of bone powder (≤ 5 mg) was collected from the coracoid found among the faunal remains at Calathamet site by using a micro drill (Dremel 200). Two independent DNA extractions with the DNA IQ System (Promega) were carried out following the manufacturer's instructions. Laboratory work concerning unamplified DNA was performed in a properly equipped and specifically designated facility. Extraction and PCR blank controls were constantly incorporated to check against possible contamination. Workflow was conducted in strict conformity to ancient DNA protocols throughout all steps.

Mitochondrial DNA amplification and sequencing

A 185 bp-long fragment of the mtDNA Control Region (pos. 151-335 of HE793456 GenBank sequence, see ref. 17) was amplified from modern and archival francolins (n = 5 + 77 = 82) using primers CRFra58 (forward: 5'-GTATACGTACTAAACCCA TTAT-3') and CRFra355 (reverse: 5'-TCCGATCAATAAATCCATCTGG-3') in a single PCR.

Reactions (50 µl) were prepared with 1 µl of Ampli*Taq* Gold DNA Polymerase (1 U/µl, Applied Biosystems), 4 µl 25 mM $MgCl_2$ (Applied Biosystems), 5 µl of 1x PCR Gold buffer (Applied Biosystems), 5 µl 2.5 mM dNTP (Sigma Aldrich), 3 µl of each primer (1 µM) and *ca*. 20 ng of DNA template. PCRs were run with the following thermal profile: 10 min at 94° C; then, 50 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s; final extension, 72 °C for 10 min. PCR products were purified using the Genelute PCR Clean-up Kit (final volume 40 µl; Sigma Aldrich) and directly sequenced on both DNA strands (BigDye Terminator v. 3.1 Cycle Sequencing Kit, ABI 3730 DNA automated sequencer, Applied Biosystems) at Genechron (ENEA, Rome, Italy).

Mitochondrial DNA analyses

An alignment was produced with CLUSTALX (v. 1.81) (57) relying on the 185-bp long fragment and employing all of the newly amplified sequences plus those already obtained in Forcina *et al.* (17) (n = 5 + 77 + 200 = 282). Haplotype composition, diversity (*h*) as well the number of pairwise differences among haplotypes (*k*) were inferred using DNASP (v. 5.00) (58). Bayesian phylogenetic analysis with Metropolis-coupled Markov chain Monte Carlo algorithms was conducted using MRBAYES (v. 3.1.2) (59) and setting HE793492 *Francolinus pictus* (painted francolin) sequence as outgroup (see 18). We used MRMODELTEST (v. 2.3) (60) to estimate the best substitution model fitting to our mtDNA dataset. Both Akaike Information Criterion (AIC = 1981.6) and Hierarchical Likelihood RatioTests ($-\ln L = 981.8$) indicated General Time Reversible (GTR) + G ($\alpha = 0.1681$) model. In a Bayesian analysis, however, the Markov chain integrates over the uncertainty in parameter values. Hence, we did not include the estimated parameter values, yet only the general form of the model. Two independent runs of analysis were conducted for 6,000,000 generations with a sample frequency of 100 (four chains, heating = 0.2, random starting tree). Convergence between the two runs was monitored in MRBAYES through the standard deviation of split frequencies, and runs were continued until this value dropped to 0.0082. We monitored with TRACER (v. 1.5) (61) the convergence of each run towards stationarity, which was reached after 1,200,000 generations. Hence, 12,000 trees were discarded as burn-in and 96,002 retained to produce 50% majority-rule consensus trees. A haplotype network was also constructed using DNA ALIGNMENT (v. 1.3.3.2, 2003-2013 Fluxus Technology) and the Median Joining method (62) as implemented in NETWORK (v. 4.6.1.2, 2004-2014 Fluxus

Technology). Mutated positions were weighted uniformly, while the epsilon tolerance parameter and the transitions/transversions *ratio* were set to 0 and 1, respectively.

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FIGURE AND TABLE LEGENDS

Fig. 1. Distribution map and sampling localities of *F. francolinus*. (A) Current range with broken lines marking out the boundaries among pairs of morphological subspecies as inferred in Fig. 2 (see also Fig. S4 and ref. 18). We painted *francolinus-arabistanicus* group in light pink, *bogdanovi-henrici* in light green, and *asiae-melanonotus* in light blue (cf., Fig. S3). Black solid circles and black crosses refer to modern and archival sampling localities. (B) Historical range (white) of the species in the Mediterranean. Archival sampling localities are indicated by a cross with the same color used in part A for the range of each pairs of subspecies. Extinction dates are reported for the populations inhabiting the western Mediterranean.[One column, Color]

Fig. 2. Bayesian phylogenetic tree computed by MRBAYES for the aligned black francolin CR haplotypes (H1-H72) and using *F. pictus* as outgroup. Posterior probability values computed in the analysis are reported for the two main nodes. Haplotypes hold by black francolins (bold) introduced into West Mediterranean are indicated by solid black arrows. Morphological subspecies pairs are given by the same colors used in Fig. 1. [One column, Color]

Electronic supplementary content

Fig. S1. The black francolin (*F. francolinus asiae*): male (left) and female (right). Photos by F. Barbanera (2008).

Fig. S2 Fresco entitled "The hunters gathering" by the Flemish painter J. Sustermans (Palatine Gallery, Florence, 17th century). The character on the right holds a male black francolin.

Fig. S3. A few historical commercial routes connecting the Far East to the Mediterranean are shown. The Silk Road (black-red line) was begun in the 2nd century BC and survived until at least the 15th century, about 150 years after Marco Polo, when the travelling along the sea became prevailing: the yellow-red line shows the spice trade route during the Middle Age. The capture of Goa and Malacca (*ca*. 1510) as well as the establishment of first trading posts in the Moluccas (Spice Islands) gave Portugal the monopoly over trade in the Indian Ocean (yellow-white line or Portuguese route).

Fig. S4. Black francolin haplotype network drawn on the basis of the 185-bp long CR fragment and the whole (modern + archival) sample size. A scale to infer the number of haplotypes (1-72) for each pie is provided together with a length bar to compute the number of mutational changes. The color of each country is indicated as well as the number of each haplotype (Table S1). The three mitochondrial DNA lineages corresponding to three pairs of subspecies are indicated as well. For the sake of clarity, the *bogdanovi-henrici* group was separated from *francolinus-arabistanicus* and *asiae-melanonotus* group by seven and six mutational steps, respectively.

Table S1. Sample details relative to the sample size employed in this study. Data include country (with region/province/district), locality with latitude/longitude (Lat/Long), number of sample, type of tissue, year of collection, museum name, specimen voucher, CR mtDNA haplotype, and literature record. Legend: *, Turkish occupied area of the island of Cyprus; ** West Bank, Palestinian Territory; Res., Reserve; San., Sanctuary;***, out of present-day range of the species; Wild., Wildlife; c., century; **#**, at first labeled as *F. francolinus*, this sample was later assigned to the taxon *Alectoris graeca whitakeri*: see Results.

Table S2. GenBank accession codes for the mtDNA CR haplotypes of the of this study (1-72: *Francolinus francolinus*; 73: *Alectoris graeca*)

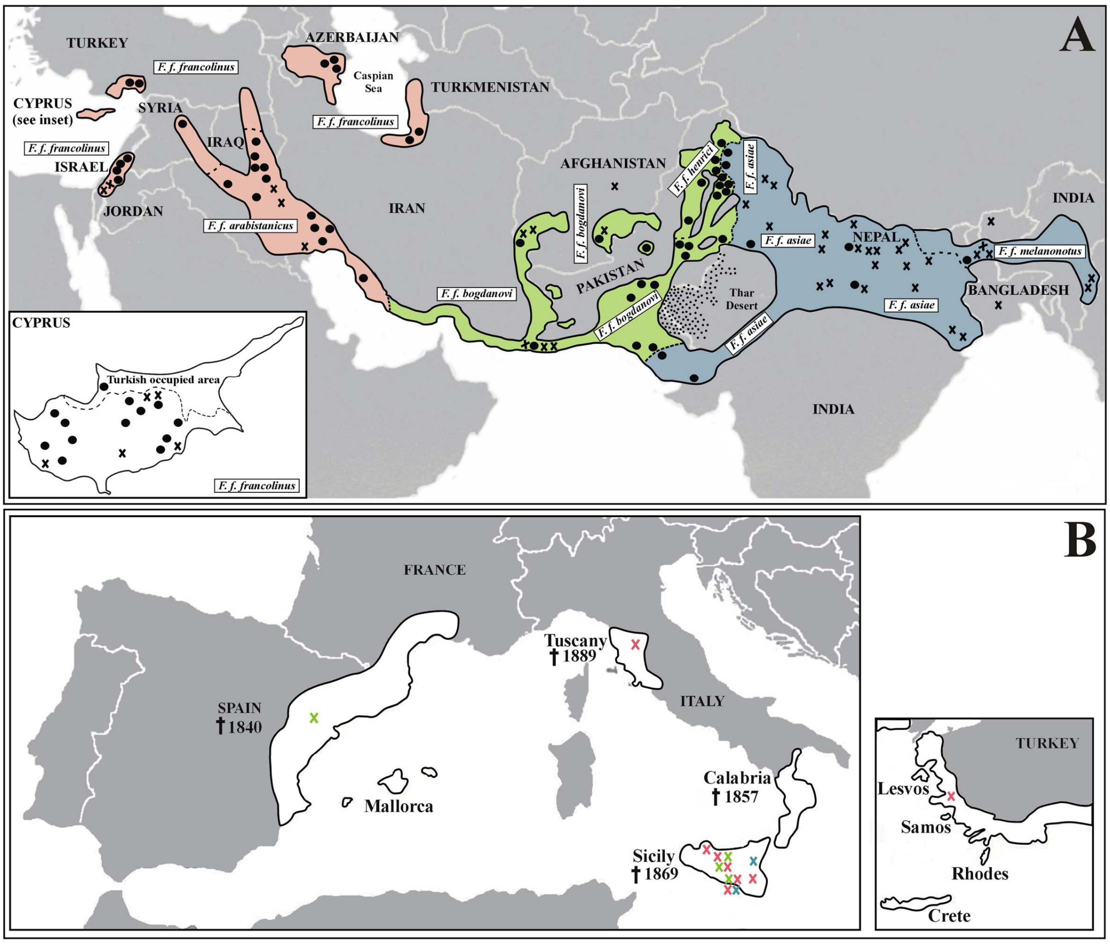
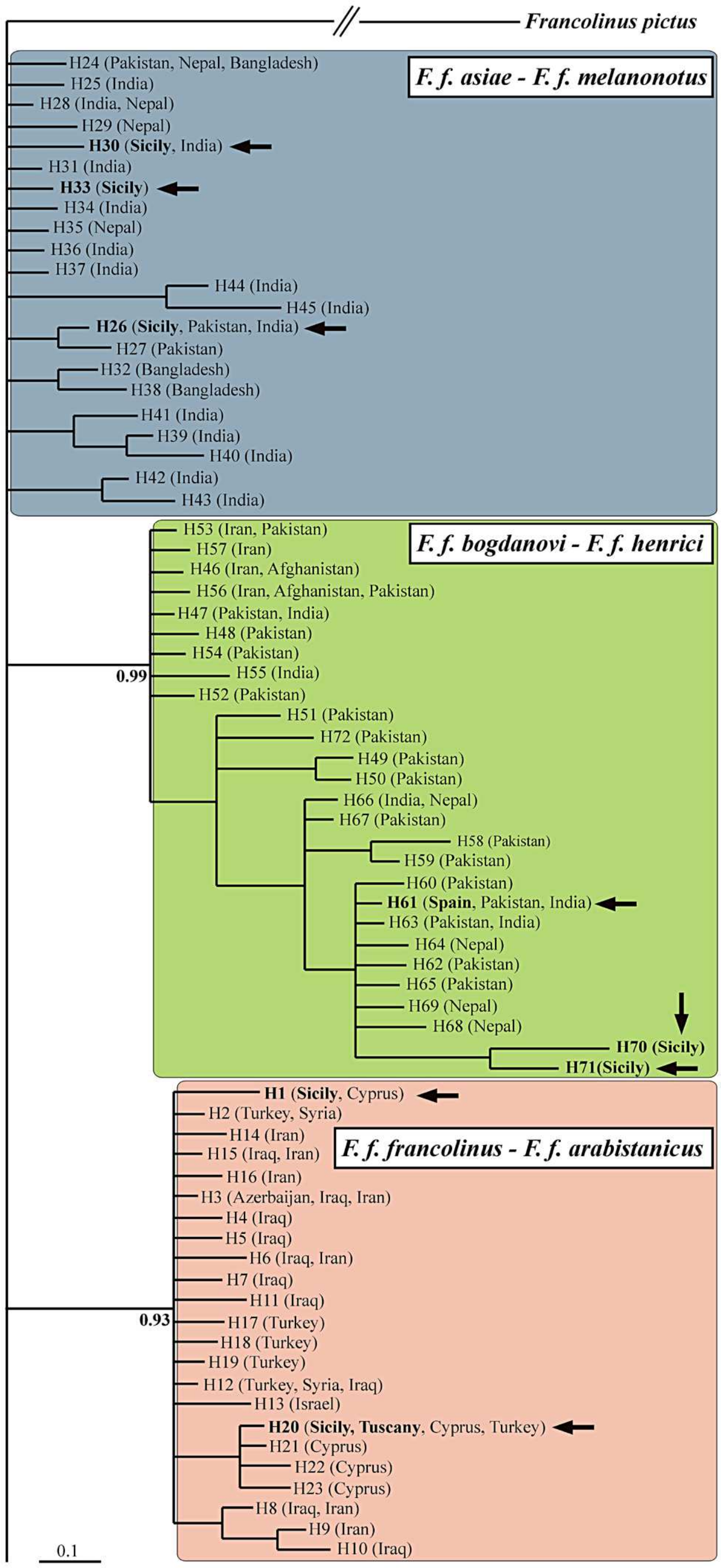

A
TURKEY
F. f. francolinus
AZERBAIJAN
Caspian Sea
TURKMENISTAN
F. f. francolinus
CYPRUS (see inset)
SYRIA
F. f. francolinus
IRAQ
ISRAEL
JORDAN
F. f. arabistanicus
IRAN
AFGHANISTAN
F. f. bogdanovi
F. f. henrici
F. f. asiae
PAKISTAN
F. f. bogdanovi
F. f. bogdanovi
Thar Desert
F. f. asiae
NEPAL
F. f. asiae
F. f. melanonotus
INDIA
BANGLADESH
F. f. asiae
INDIA
CYPRUS
Turkish occupied area
F. f. francolinus
B
FRANCE
Tuscany
† 1889
SPAIN
† 1840
ITALY
Mallorca
Calabria
† 1857
Sicily
† 1869
TURKEY
Lesvos
Samos
Rhodes
Crete

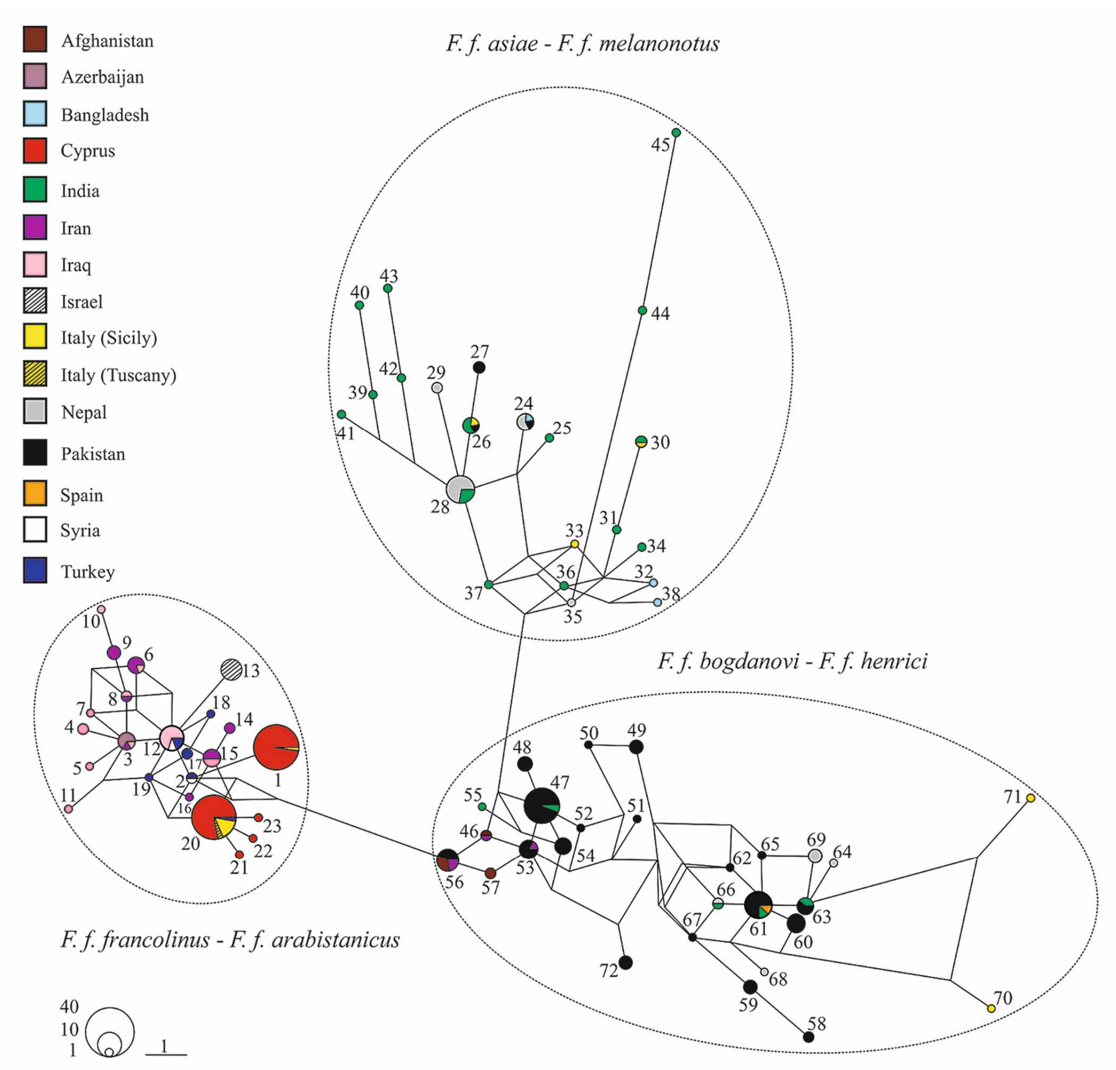
Figure 1


Francolinus pictus
F. f. asiae - F. f. melanonotus
H24 (Pakistan, Nepal, Bangladesh)
H25 (India)
H28 (India, Nepal)
H29 (Nepal)
H30 (Sicily, India)
H31 (India)
H33 (Sicily)
H34 (India)
H35 (Nepal)
H36 (India)
H37 (India)
H44 (India)
H45 (India)
H26 (Sicily, Pakistan, India)
H27 (Pakistan)
H32 (Bangladesh)
H38 (Bangladesh)
H41 (India)
H39 (India)
H40 (India)
H42 (India)
H43 (India)
F. f. bogdanovi - F. f. henrici
H53 (Iran, Pakistan)
H57 (Iran)
H46 (Iran, Afghanistan)
H56 (Iran, Afghanistan, Pakistan)
H47 (Pakistan, India)
H48 (Pakistan)
H54 (Pakistan)
0.99
H55 (India)
H52 (Pakistan)
H51 (Pakistan)
H72 (Pakistan)
H49 (Pakistan)
H50 (Pakistan)
H66 (India, Nepal)
H67 (Pakistan)
H58 (Pakistan)
H59 (Pakistan)
H60 (Pakistan)
H61 (Spain, Pakistan, India)
H63 (Pakistan, India)
H64 (Nepal)
H62 (Pakistan)
H65 (Pakistan)
H69 (Nepal)
H68 (Nepal)
H70 (Sicily)
H71(Sicily)
F. f. francolinus - F. f. arabistanicus
H1 (Sicily, Cyprus)
H2 (Turkey, Syria)
H14 (Iran)
H15 (Iraq, Iran)
H16 (Iran)
H3 (Azerbaijan, Iraq, Iran)
H4 (Iraq)
H5 (Iraq)
H6 (Iraq, Iran)
H7 (Iraq)
H11 (Iraq)
0.93
H17 (Turkey)
H18 (Turkey)
H19 (Turkey)
H12 (Turkey, Syria, Iraq)
H13 (Israel)
H20 (Sicily, Tuscany, Cyprus, Turkey)
H21 (Cyprus)
H22 (Cyprus)
H23 (Cyprus)
H8 (Iraq, Iran)
H9 (Iran)
H10 (Iraq)
0.1

784 **Figure 2**

Figure S1

797 **Figure S2**



798 **Figure S3**

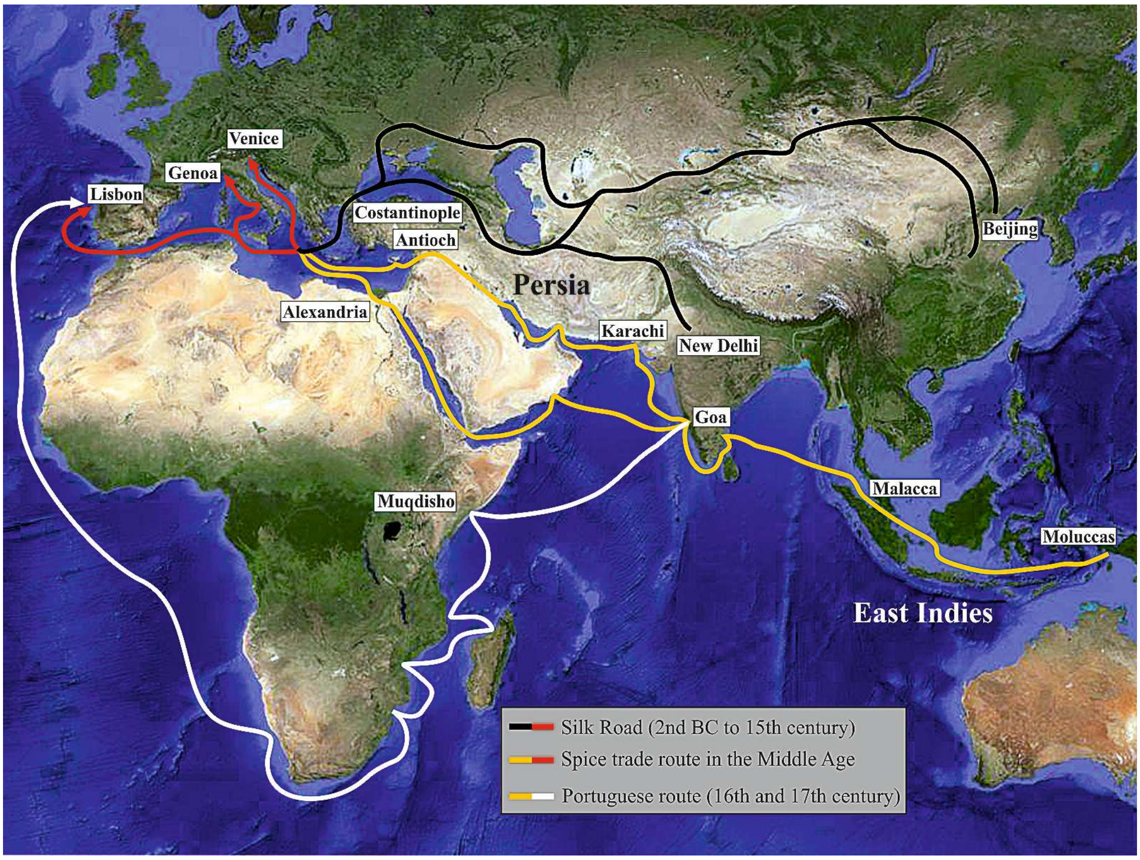

Venice
Genoa
Lisbon
Costantinople
Antioch
Beijing
Persia
Alexandria
Karachi
New Delhi
Goa
Muqdisho
Malacca
Moluccas
East Indies
Silk Road (2nd BC to 15th century)
Spice trade route in the Middle Age
Portuguese route (16th and 17th century)

Figure S4

Table S1. Sample details relative to the complete black francolin sample size employed in this study.

Country	Region/Province/District	Locality	Lat/Long	Sample (n)	Tissue	Year(s)	Museum (City; voucher)	CR haplotype (185 bp)	Literature record
Modern samples									
Afghanistan	Kandahar	Near Kandahar city	31°36'N; 65°42'E	1	Feather	2010	-	46	Forcina et al. (2012)
Azerbaijan	Sabirabad Rayon	Sabirabad	40°03'N; 48°48'E	1	Feather	2011	-	3	Forcina et al. (2012)
	Imishli Rayon	Imishli	39°87'N; 48°07'E	1	Feather	2011	-	3	Forcina et al. (2012)
	Lankaram Rayon	Gizil-Agach State Reserve	39°18'N; 48°96'E	2	Feather	2011	-	3	Forcina et al. (2012)
Bangladesh	Tetulia	1 Km East of Indian border	26°29'N; 88°20'E	2	Feather	2009	-	24,32	Forcina et al. (2012)
Cyprus	Paphos	Lysos	35°00'N; 32°51'E	4	Liver	2007-2008-2010	-	1	Forcina et al. (2012)
	Paphos	Yiolou	34°92'N; 32°47'E	7	Liver	2007-2008	-	1	Forcina et al. (2012)
	Paphos	Polemi	34°88'N; 32°51'E	9	Liver	2007-2009	-	1	Forcina et al. (2012)
	Paphos	Makounta	34°05'N; 32°49'E	3	Liver	2007-2010	-	1	Forcina et al. (2012)
	Paphos	Psathi	34°90'N; 32°53'E	7	Liver	2007	-	1	Forcina et al. (2012)
	Nicosia	Peristerona	35°13'N; 33°08'E	6	Liver	2007-2009	-	20	Forcina et al. (2012)
	Nicosia	Akaki	35°13'N; 33°13'E	2	Liver	2007	-	20	Forcina et al. (2012)
	Nicosia	Dali	35°03'N; 33°43'E	8	Liver	2007	-	20,21	Forcina et al. (2012)
	Nicosia	Astromeritis	35°14'N; 33°03'E	3	Liver	2007-2009	-	20	Forcina et al. (2012)
	Nicosia	Ayia Marina	35°22'N; 33°12'E	2	Liver	2007	-	20	Forcina et al. (2012)

	Nicosia	Sha	34°94'N; 33°39'E	1	Liver	2007	-	20	Forcina et al. (2012)
	Nicosia	Potamia	35°04'N; 33°44'E	6	Liver	2007	-	20,22	Forcina et al. (2012)
	Nicosia	Gaziveran*	35°17'N; 32°90'E	1	Liver	2011	-	20	Forcina et al. (2012)
India	Gujarat, Kachchh	Near Bhuj	Unknown	3	Feather	2011	-	26	Forcina et al. (2012)
	Uttar Pradesh	Sultanpur Wild. Bird San.*	26°25'N; 82°06'E	1	Feather	2011	-	47	Forcina et al. (2012)
	Rajastan	Bikaner	28°01'N; 73°18'E	2	Feather	2013	-	28	This study
Iran	Kuzestan	Karoon Dam	80 Km North of Awhaz	1	Feather	2009	-	15	Forcina et al. (2012)
	Kuzestan	Abadan	110 Km South of Awhaz	1	Feather	2009	-	6	Forcina et al. (2012)
	Kuzestan	Ramhormoz	150 Km East of Awhaz	1	Feather	2009	-	15	Forcina et al. (2012)
	Kuzestan	Ahwaz	31°50'N; 48°00'E	7	Feather	2009	-	6,8,14,16	Forcina et al. (2012)
	Bushehr	Heleh Protected Area	29°09'N; 50°50'E	3	Feather	2010	-	9	Forcina et al. (2012)
	Sistan and Baluchistan	Zabol	31°02'N; 61°30'E	2	Feather	2009	-	46,53	Forcina et al. (2012)
	Sistan and Baluchistan	Hamoun Protected Wetland	31°20'N; 60°50'E	2	Feather	2011	-	56	Forcina et al. (2012)
	Mazandaran	Near Sari	Unknown	1	Feather	2010	-	3	Forcina et al. (2012)
	Golestan	Near Gorgan	Unknown	1	Feather	2010	-	15	Forcina et al. (2012)
Iraq	Al Anbar	Al Asad	33°47'N; 42°26'E	2	Feather	2009	-	15	Forcina et al. (2012)
	Diyala	Al Attariyah	33°31'N; 44°45'E	5	Feather	2009-2010	-	7,12,15	Forcina et al. (2012)
	Al Taamim	Kirkuk	35°30'N; 43°51'E	2	Feather	2009	-	12	Forcina et al. (2012)

	Salah Aldin	Al Alam	34°38'N; 43°42'E	1	Feather	2009	-	12	Forcina et al. (2012)
	Salah Aldin	Al Mahzam	34°43'N; 43°40'E	1	Feather	2009	-	4	Forcina et al. (2012)
	Anbar	Hawijat Albu Alwan	33°26'N; 43°34'E	1	Feather	2009	-	12	Forcina et al. (2012)
	Salah Aldin	Gelaat Albu Ageel	34°37'N; 43°47'E	2	Feather	2009	-	6,11	Forcina et al. (2012)
Israel	Mehoz Ha Tzafon	Poriya	32°44'N; 35°30'E	1	Feather	2008	-	13	Forcina et al. (2012)
	Samaria	Tel Al Beida	32°21'N; 35°31'E	1	Feather	2009	-	13	Forcina et al. (2012)
	Mehoz Ha Tzafon	Nimrod	33°10'N; 35°38'E	1	Feather	2007	-	13	Forcina et al. (2012)
	Mehoz Ha Tzafon	Nazareth	32°35'N; 35°24'E	1	Feather	2008	-	13	Forcina et al. (2012)
	Mehoz Ha Tzafon	Kafr Kama	32°43'N, 35°27'E	1	Feather	2009	-	13	Forcina et al. (2012)
Nepal	Western Terai	Unknown	Unknown	14	Feather	2008	-	24,28,29	Forcina et al. (2012)
Pakistan	Sindh	Badin	24°39'N; 68°50'E	16	Feather	2009	-	26,27,47,52,58 59,62,63,65	Forcina et al. (2012)
	Sindh	Near Karachi	24°51'N; 67°00'E	4	Feather	2010	-	47,48,60	Forcina et al. (2012)
	Baluchistan	Dasht River, near Jiwani	25°03'N; 61°45'E	2	Feather	2009	-	49	Forcina et al. (2012)
	Sindh	Near Larkana	27°55'N; 68°22'E	5	Feather	2010	-	47,53,60	Forcina et al. (2012)
	Sindh	Ghotki	28°01'N; 69°19'E	3	Feather	2008	-	47,67	Forcina et al. (2012)
	Sindh	Jacobabad	28°17'N; 68°26'E	2	Feather	2009	-	47,58	Forcina et al. (2012)
	Punjab, Muzaffar Garh	Alipur	29°23'N; 70°18'E	3	Feather	2008	-	47	Forcina et al. (2012)
	Punjab, Bahawalnagar	Haroon Abad	29°37'N; 73°08'E	5	Feather	2008	-	54	Forcina et al. (2012)

	Baluchistan, Barkhan	Rakhni	30°03'N; 69°55'E	2	Feather	2008	-	47,61	Forcina et al. (2012)
	Punjab, Muzaffar Garh	Ghazi Ghat	30°06'N; 70°80'E	1	Feather	2008	-	47	Forcina et al. (2012)
	Punjab, Dera Ghazi Khan	Bait Suvai	30°45'N; 70°89'E	13	Feather	2008	-	47,48,51,60,63	Forcina et al. (2012)
	Punjab, Chawkal	Rabal	32°56'N; 72°52'E	6	Feather	2008	-	61	Forcina et al. (2012); this study
	Azad Jammu & Kashmir	Sialkot	32°35'N; 74°30'E	6	Feather	2009	-	47,53,56,63	Forcina et al. (2012)
	Azad Jammu & Kashmir	Bhimbar	32°58'N; 74°02'E	1	Feather	2009	-	60	Forcina et al. (2012)
	Azad Jammu & Kashmir	Mirpur	33°08'N; 73°44'E	1	Feather	2009	-	61	Forcina et al. (2012)
	Azad Jammu & Kashmir	Nikyal, near Kotli	33°28'N; 74°06'E	1	Feather	2008	-	56	Forcina et al. (2012)
	Azad Jammu & Kashmir	Kohala	34°12'N; 73°29'E	3	Feather	2009	-	24,61,72	Forcina et al. (2012)
	Azad Jammu & Kashmir	Tandali	34°13'N; 73°29'E	1	Feather	2009	-	61	Forcina et al. (2012)
	Azad Jammu & Kashmir	Muzaffarabad	34°22'N; 73°28'E	2	Feather	2009	-	72	Forcina et al. (2012)
Syria	Ar-Rakkah	Near Ar-Rakkah city	36°00'N; 38°30'E	2	Feather	2010	-	2,12	Forcina et al. (2012)
Turkey	Mersin	Tarsus	36°92'N; 34°90'E	2	Feather	2010	-	2,17	Forcina et al. (2012); this study
Sub-total				**205**					
Archival samples									
Afghanistan	Ghor***	Kamran	34°64'N; 66°30'E	1	Toe pad	1885	Natural History Museum (Tring; BMNH 1886.9.16.204)	56	This study
	Farah	Helmand River	31°50'N; 61°50'E	1	Toe pad	1926	Natural History Museum (Tring; BMNH 1886.M.2008)	56	This study
	Farah	Helmand River	31°50'N; 61°50'E	1	Toe pad	1926	Natural History Museum (Tring; BMNH 1886.M.2010)	56	This study

	Kandahar	Near Kandahar city	31°36'N; 65°42'E	1	Toe pad	1905	American Natural History Museum (New York; AMNH 464992)	57	This study
	Kandahar	Near Kandahar city	31°36'N; 65°42'E	1	Toe pad	1905	American Natural History Museum (New York; AMNH 464993)	57	This study
Bangladesh	Dhaka***	Dhaka	23°07'N; 90°40'E	1	Toe pad	1871	Natural History Museum (Tring; BMNH 1889.5.10.1339)	38	This study
Cyprus	Nicosia	Nicosia	35°11'N; 33°40'E	1	Toe pad	1875	Natural History Museum (Tring; BMNH 1888.7.26.176)	23	This study
	Nicosia	Nicosia	35°11'N; 33°40'E	1	Toe pad	1878	Natural History Museum (Tring; BMNH 1888.7.26.177)	1	This study
	Nicosia	Nicosia	35°11'N; 33°40'E	1	Toe pad	1878	Natural History Museum (Tring; BMNH 1888.7.26.175)	1	This study
	Nicosia	Nicosia	35°11'N; 33°40'E	1	Toe pad	1875	Natural History Museum (Tring; BMNH 1888.7.26.181)	20	This study
	Nicosia	Nicosia	35°11'N; 33°40'E	1	Toe pad	1888	Natural History Museum (Tring; BMNH 1888.7.26.178)	20	This study
	Nicosia	Nicosia	35°11'N; 33°40'E	1	Toe pad	1889	Natural History Museum (Tring; BMNH 1888.7.26.184)	1	This study
	Nicosia	Nicosia	35°11'N; 33°40'E	1	Toe pad	1875	Natural History Museum (Tring; BMNH 1888.7.26.174)	20	This study
	Nicosia	Lefka*	35°10'N; 32°85'E	1	Toe pad	1878	Natural History Museum (Tring; BMNH 1888.7.26.180)	1	This study
	Nicosia	Lefka*	35°10'N; 32°85'E	1	Toe pad	1878	Natural History Museum (Tring; BMNH 1888.7.26.183)	1	This study
	Nicosia	Lefka*	35°10'N; 32°85'E	1	Toe pad	1878	Natural History Museum (Tring; BMNH 1888.7.26.182)	1	This study
	Paphos	Paphos	34°76'N; 32°41'E	1	Toe pad	1909	Natural History Museum (Tring; BMNH 1909.11.30.29)	1	This study
	Unknown	Unknown	Unknown	1	Toe pad	1886	Hunterian Museum, Zoology Section (Glasgow; GLAHM 107509)	1	This study
Iraq	Basrah Governorate	Basrah	30°50'N; 47°81'E	1	Toe pad	1917	Natural History Museum (Tring; BMNH 1941.5.30.2940)	3	This study
	Basrah Governorate	Basrah	30°50'N; 47°81'E	1	Toe pad	1917	Natural History Museum (Tring; BMNH 1941.5.30.2938)	10	This study
	Basrah Governorate	Al Faw	29°98'N; 48°46'E	1	Toe pad	1921	Natural History Museum (Tring; BMNH 1924.3.20.29)	12	This study

	Dhi Qar Governorate	Nasiriyah	31°05'N; 46°25'E	1	Toe pad	1923	Natural History Museum (Tring; BMNH 1965.M.1999)	8	This study
	Baghdad Governorate	Baghdad	33°25'N; 44°33'E	1	Toe pad	1954	Yale Peabody Museum (New Haven; YPM 98099)	5	This study
	Baghdad Governorate	Baghdad	33°25'N; 44°33'E	1	Toe pad	1954	Yale Peabody Museum (New Haven; YPM 98100)	4	This study
India	West Bengal, Jalpaiguri District	Birpara	26°66'N; 89°20'E	1	Toe pad	1930	Natural History Museum (Tring; BMNH 1949.whi.1.6314)	30	This study
	West Bengal, Jalpaiguri District	Birpara	26°66'N; 89°20'E	1	Toe pad	1932	Natural History Museum (Tring; BMNH 1949.whs.1.6315)	34	This study
	Manipur	Kangpokpi	25°13'N; 93°95'E	1	Toe pad	1952	Natural History Museum (Tring; BMNH 1965.M.2012)	44	This study
	Punjab	Mallanwalla (Firozpur)	31°05'N; 74°82'E	1	Toe pad	1935	Natural History Museum (Tring; BMNH 1949.25.445)	55	This study
	Manipur	Wangoo	24°38'N; 93°85'E	1	Toe pad	1896	Natural History Museum (Tring; BMNH 1895.7.14.734)	45	This study
	Bihar	Maunbhoon	25°93'N; 86°40'E	1	Toe pad	1865	Natural History Museum (Tring; BMNH 1889.5.13.321)	25	This study
	West Bengal	Siliguri	26°70'N; 88°33'E	1	Toe pad	1926	Natural History Museum (Tring; BMNH 1965.M.2011)	36	This study
	Uttar Pradesh	Sultanpur Wild. Bird San.	26°25'N; 82°06'E	1	Toe pad	1977	Natural History Museum (Tring; BMNH 1889.5.10.1317)	28	This study
	Uttar Pradesh	Agra	27°18'N; 78°02'E	1	Toe pad	1937	American Natural History Museum (New York; AMNH 776808)	42	This study
	Uttar Pradesh	Agra	27°18'N; 78°02'E	1	Toe pad	1937	American Natural History Museum (New York; AMNH 776809)	43	This study
	Uttarakhand	Kumaon	29°60'N; 79°70'E	1	Toe pad	1951	Yale Peabody Museum (New Haven; YPM 15798)	39	This study
	Himachal Pradesh	Kullu	31°98'N; 77°10'E	1	Toe pad	1922	Yale Peabody Museum (New Haven; YPM 42075)	66	This study
	Himachal Pradesh	Dharamshala	32°23'N; 76°40'E	1	Toe pad	1922	Yale Peabody Museum (New Haven; YPM 42076)	63	This study
	Assam, Goalpara District	Unknown	Unknown	1	Toe pad	1905	Yale Peabody Museum (New Haven; YPM 42077)	31	This study
	Uttarakhand	Fern Hill (Lohaghat)	29°25'N; 80°06'E	1	Toe pad	1908	Yale Peabody Museum (New Haven; YPM 42078)	63	This study

	West Bengal	Kharagpur	22°33'N; 87°32'E	1	Toe pad	1905	University of Michigan Museum of Zoology (Ann Arbor; UMMZ 114992)	28	This study
	West Bengal	Kharagpur	22°33'N; 87°32'E	1	Toe pad	1905	University of Michigan Museum of Zoology (Ann Arbor; UMMZ 114993)	41	This study
	Uttar Pradesh	Mussorie	30°45'N; 78°08'E	1	Toe pad	1905	University of Michigan Museum of Zoology (Ann Arbor; UMMZ 234290)	61	This study
	Uttar Pradesh	Gorakhpur	26°45'N; 83°22'E	1	Toe pad	1919	Field Museum of Natural History (Chicago; FNHM 402182)	37	This study
	Bihar	Khapkat	26°22'N; 84°36'E	1	Toe pad	1948	Field Museum of Natural History (Chicago; FNHM 420349)	40	This study
Israel	Jericho Governorate	Jericho**	31°83'N; 35°43'E	1	Toe pad	1918	Natural History Museum (Tring 1965.M.1991)	13	This study
	Jericho Governorate	Jericho**	31°83'N; 35°52'E	1	Toe pad	1923	Natural History Museum (Tring 1965.M.1997)	13	This study
	Jericho Governorate	Wadi Quelt**	31°83'N; 35°42'E	1	Toe pad	1945	Natural History Museum (Tring 1946.63.21)	13	This study
Italy	Sicily***	Unknown	Unknown	1	Toe pad	1834	Senckenberg Natural History Museum (Frankfurt; SMF 23598)	26	This study
	Sicily***	Unknown	Unknown	1	Toe pad	Before 1845	Royal Belgian Institute of Natural Sciences (Bruxelles; RBINS 3098 B)	20	This study
	Sicily***	Unknown	Unknown	1	Toe pad	Before 1845	Royal Belgian Institute of Natural Sciences (Bruxelles; RBINS 3098 D)	20	This study
	Sicily***	Catania	37°30'N; 15°05'E	1	Feather	1843	Municipal Museum of Zoology (Rome; A.d.O 1834)	70	This study
	Sicily***	Gela	37°04'N; 14°15'E	1	Toe pad	Before 1865	Municipal Museum of Natural Sciences (Randazzo)	30	This study
	Sicily***	Unknown	Unknown	1	Toe pad	Before 1865	Regional Museum of Natural History (Terrasini; N. inv. R.S. 2392)	33	This study
	Sicily***	Gela	37°04'N; 14°15'E	1	Toe pad	1866-1869	Doderlein Museum of Zoology (Palermo; AV 662)	71	This study
	Sicily***	Gela	37°04'N; 14°15'E	1	Toe pad	1866-1869	Doderlein Museum of Zoology (Palermo; AV 663)	1	This study
	Sicily***	Gela	37°04'N; 14°15'E	1	Feather	1854	Museum of Zoology and Natural History (Florence; N Coll. 7995)	20	This study
	Sicily***	Caltagirone	37°14'N; 14°31'E	1	Feather	1850	Museum of Zoology and Natural History (Florence; N. Coll 8000)	20	This study

	Sicily***	Palermo	38°06'N; 13°21'E	1	Feather	1862	Museum of Zoology and Natural History (Florence; N. Coll 8001)	20	This study
	Sicily***	Castellammare del Golfo#	37°67'N; 12°79'E	1	Bone	Second half 13th c.	Doderlein Museum of Zoology (Palermo; -)	73	This study
	Tuscany***	Carmignano	43°49'N; 11°01'E	1	Feather	First half 19th c.	Museum of Zoology and Natural History (Florence; N. Coll 7996)	20	This study
Nepal	Karnali	Darma	29°73'N; 82°08'E	1	Toe pad	1936	Natural History Museum (Tring;1938.7.15.22)	64	This study
	Narayani	Katunjie	27°55'N; 84°26'E	1	Toe pad	1935	Natural History Museum (Tring;1938.7.15.21)	69	This study
	Bagmati	Nepal Valley	27°66'N; 85°35'E	1	Toe pad	1877	Natural History Museum (Tring;1889.5.10.1267)	69	This study
	Lumbini	Tribeni	27°45'N; 83°92'E	1	Toe pad	1935	Natural History Museum (Tring;1938.7.15.23)	35	This study
	Rapti	Rapti Valley	28°20'N; 82°30'E	1	Toe pad	1962	Bavarian State Collection of Zoology (Munich; ZSM 239)	68	This study
	Rapti	Rapti Valley	28°20'N; 82°30'E	1	Toe pad	1962	Bavarian State Collection of Zoology (Munich; ZSM 240)	66	This study
	Bagmati	Trishuli	27°55'N; 85°15'E	1	Toe pad	1967	Field Museum of Natural History (Chicago; FNHM 424698)	69	This study
Pakistan	Baluchistan	Makran coast	25°21'N; 61°65'E	1	Toe pad	?	Natural History Museum (Tring;1908.12.20.11)	49	This study
	Baluchistan	Unknown	Unknown	1	Toe pad	1911	Bavarian State Collection of Zoology (Munich; ZSM 297)	59	This study
	Baluchistan	Unknown	Unknown	1	Toe pad	1911	Bavarian State Collection of Zoology (Munich; ZSM 537)	50	This study
Turkey	Hatay Province	Lake of Antioch	36°18' N; 36°18'E	1	Toe pad	1933	Natural History Museum (Tring 1965.M.2013)	12	This study
	Hatay Province	Lake of Antioch	36°18'N; 36°18'E	1	Toe pad	1933	Natural History Museum (Tring 1965.M.2014)	12	This study
	Adana Province	Adana	37°00'N; 35°19'E	1	Toe pad	1879	Natural History Museum (Tring 1896.1.1.536)	18	This study
	Mersin Province	Mersin	36°80'N; 34°63'E	1	Toe pad	1908	Municipal Museum of Zoology (Rome; A.d.O 16505)	19	This study
	Izmir Province***	Izmir	38°42'N; 27°13'E	1	Toe pad	?	Bavarian State Collection of Zoology (Munich; ZSM SMYRNE)	20	This study

	Adana Province	Adana	37°00'N; 35°19'E	1	Toe pad	?	Bavarian State Collection of Zoology (Munich; ZSM 4.50)	17	This study
Spain	unknown	Unknown	Unknown	1	Toe pad	1838	Natural History Museum (Geneva; MHNG 701.083)	61	This study
Sub-total				**77**					
Total				**282**					

Sample details relative to the sample size employed in this study. Data include country (with region/province/district), locality with latitude/longitude (Lat/Long), number of sample, type of tissue, year of collection, museum name, specimen voucher, CR mtDNA haplotype, and literature record. Legend: *, Turkish occupied area of the island of Cyprus; ** West Bank, Palestinian Territory; Res., Reserve; San., Sanctuary; ***, out of present-day range of the species; Wild., Wildlife; c., century; **#**, at first labeled as *F. francolinus*, this sample was later assigned to the taxon *Alectoris graeca whitakeri*: see Results.

Table S2. GenBank accession codes for the mtDNA CR haplotypes of the of this study (1-72: *Francolinus francolinus*; 73: *Alectoris graeca*)

Haplotype (H)	GenBank accession code	Haplotype (H)	GenBank accession code	Haplotype (H)	GenBank accession code
1	LK871783	25	LK871807	49	LK871831
2	LK871784	26	LK871808	50	LK871832
3	LK871785	27	LK871809	51	LK871833
4	LK871786	28	LK871810	52	LK871934
5	LK871787	29	LK871811	53	LK871835
6	LK871788	30	LK871812	54	LK871836
7	LK871789	31	LK871813	55	LK871837
8	LK871790	32	LK871814	56	LK871838
9	LK871791	33	LK871815	57	LK871839
10	LK871792	34	LK871816	58	LK871840
11	LK871793	35	LK871817	59	LK871841
12	LK871794	36	LK871818	60	LK871842
13	LK871795	37	LK871819	61	LK871843
14	LK871796	38	LK871820	62	LK871844
15	LK871797	39	LK871821	63	LK871845
16	LK871798	40	LK871822	64	LK871846
17	LK871799	41	LK871823	65	LK871847
18	LK871800	42	LK871824	66	LK871848
19	LK871801	43	LK871825	67	LK871849
20	LK871802	44	LK871826	68	LK871850
21	LK871803	45	LK871827	69	LK871851
22	LK871804	46	LK871828	70	LK871852
23	LK871805	47	LK871829	71	LK871853
24	LK871806	48	LK871830	72	LK871854
				73	LK871855