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Out-of-Africa, human-mediated dispersal of the common cat flea, *Ctenocephalides felis*: The hitchhiker's guide to world domination ☆

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ABSTRACT

The cat flea (*Ctenocephalides felis*) is the most common parasite of domestic cats and dogs worldwide. Due to the morphological ambiguity of *C. felis* and a lack of — particularly largescale — phylogenetic data, we do not know whether global *C. felis* populations are morphologically and genetically conserved, or whether human-mediated migration of domestic cats and dogs has resulted in homogenous global populations. To determine the ancestral origin of the species and to understand the level of global pervasion of the cat flea and related taxa, our study aimed to document the distribution and phylogenetic relationships of *Ctenocephalides* fleas found on cats and dogs worldwide. We investigated the potential drivers behind the establishment of regional cat flea populations using a global collection of fleas from cats and dogs across six continents. We morphologically and molecularly evaluated six out of the 14 known taxa comprising genus *Ctenocephalides*, including the four original *C. felis* subspecies (*Ctenocephalides felis felis*, *Ctenocephalides felis strongylus*, *Ctenocephalides felis orientis* and *Ctenocephalides felis damarensis*), the cosmopolitan species *Ctenocephalides canis* and the African species *Ctenocephalides connatus*. We confirm the ubiquity of the cat flea, representing 85% of all fleas collected (4357/5123). Using a multigene approach combining two mitochondrial (*cox1* and *cox2*) and two nuclear (Histone H3 and EF-1α) gene markers, as well as a *cox1* survey of 516 fleas across 56 countries, we demonstrate out-of-Africa origins for the genus *Ctenocephalides* and high levels of genetic diversity within *C. felis*. We define four bioclimatically limited *C. felis* clusters (Temperate, Tropical I, Tropical II and African) using maximum entropy modelling. This study defines the global distribution, African origin and phylogenetic relationships of global *Ctenocephalides* fleas, whilst resolving the taxonomy of the *C. felis* subspecies and related taxa. We show that humans have inadvertently precipitated the expansion of *C. felis* throughout the world, promoting diverse population structure and bioclimatic plasticity. By demonstrating the link between the global cat flea communities and their affinity for specific bioclimatic niches, we reveal the drivers behind the establishment and success of the cat flea as a global parasite.

1. Introduction

Parasites are an evolutionary success story that represent around half of all species on Earth (Windsor, 1998; Weinstein and Kuris, 2016). Domesticated cats and dogs provide a means of dispersal for the parasites that infest them, including the cat flea

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☆ Note: Nucleotide sequence data reported in this paper are available in the GenBank under accession numbers MG586243-MG586782 and the Barcode of Life Data (BOLD) Systems under accession numbers: CTENO001-18 to CTENO551-18 and ALFLE001-14 to ALFLE024-14.

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Ctenocephalides felis (Bouché, 1835) and its close relative, the dog flea, *Ctenocephalides canis* (Curtis, 1826). The cat flea is assumed to be the most globally pervasive flea species on Earth (Clark et al., 2018). Its affinity for domestic cat and dog hosts and its consequently synanthropic life history has permitted widespread geographical dispersal of the species (Hopkins and Rothschild, 1953; Rust, 2017).

An African emergence of the cat flea is assumed a priori, but the origin of the species has not been empirically investigated (Hopkins and Rothschild, 1953; Rust and Dryden, 1997). The theory assumes co-evolution of the cat flea and its dominant host, the domestic cat, *Felis silvestris catus* Linnaeus, 1758, which likely originated in Africa and the Near East from ancestral African wildcats, *Felis silvestris lybica* Forster, 1780 (Driscoll et al., 2007; Hu et al., 2014).

The cat flea species historically includes four geographically defined subspecies: the cosmopolitan *Ctenocephalides felis felis* (Bouché, 1835), an Asian subspecies *Ctenocephalides felis orientis* (Jordan, 1925) and two subspecies restricted to the African continent: *Ctenocephalides felis strongylus* (Jordan, 1925) and *Ctenocephalides felis damarensis* Jordan, 1936. Since their original description, *C. f. orientis* and *C. f. damarensis* have been morphologically reclassified as full species (*C. orientis* and *C. damarensis*, respectively), but the genetic identity of the *C. felis* subspecies remains elusive (De Meillon et al., 1961; Louw and Horak, 1995; Beaucournu and Menier, 1998; Ménier and Beaucournu, 1998). Due to morphological ambiguity and the paucity of available genetic data for taxa in the genus *Ctenocephalides* Stiles and Collins, 1930, it remains unknown whether worldwide *C. felis* populations are genetically homogenous (Beaucournu and Menier, 1998; Lawrence et al., 2014, 2015a).

Isolation of *C. felis* populations around the world was probably confounded by historical human-mediated migration of cats and dogs (Koch et al., 2016). The rapid mutation rates of mtDNA compared with nDNA enables analysis of recent divergences within and between species and can give insights into flea dispersal patterns that may reveal signatures of human migration (Avise et al., 1987; Avise, 2009). This data can also be used to reveal species origins as ancestral populations exhibit higher genetic diversity values compared with populations that have recently expanded into novel territory (Savolainen et al., 2002; Ma et al., 2012). The implementation of a combined morphological and molecular taxonomic approach to define global cat flea populations and elucidate their origins is imperative given the claims suggesting temperature-dependent biological differences between subspecies (Yao et al., 2006).

We aimed to discover the ancestral origin of the ubiquitous cat flea, as a consequence of human-mediated dispersal of domesticated cats and dogs. To achieve this, we collected and analysed the most common *Ctenocephalides* flea species and subspecies infesting cats and dogs across six continents and 56 countries. Global cat flea communities were used to clarify the taxonomic status of the four original continental *C. felis* subspecies. We assessed the efficiency of the cytochrome c oxidase I (*cox1*) mtDNA barcoding marker together with cytochrome c oxidase II (*cox2*) and two nuclear markers, histone H3 and elongation factor 1 alpha (EF-1α) for delineating *Ctenocephalides* taxa and for inferring the global population structure of *C. felis*. We show how thermal zones have shaped the distribution of the cat flea, in order to explain the widespread and climatically diverse distribution of the species.

2. Materials and methods

2.1. Specimen collection, morphological taxonomy and isolation of total DNA

Fleas were obtained from 57 countries, primarily from cat and dog hosts (Supplementary Table S1). All fleas were collected opportunistically and donated for this study by colleagues listed in Supplementary Table S2. All fleas were stored in ≥70% (v/v) ethanol, transported to the University of Sydney, Australia and identified to species level using a dissection microscope following morphological keys and descriptions (Hopkins and Rothschild, 1953; Dunnet and Mardon, 1974; Segerman, 1995; Beaucournu and Menier, 1998). The only flea from Canada was *Pulex irritans* Linnaeus, 1758; the remaining 56 countries included specimens of the genus *Ctenocephalides*.

From the initial set of *Ctenocephalides* spp. specimens ($n = 4771$ from 56 countries), a subset of fleas ($n = 572$) underwent DNA isolation (Supplementary Table S1). Some *Ctenocephalides* spp. specimens ($n = 154$) were previously characterised by us and were included in this study (Lawrence et al., 2014, 2015a, 2015b; Hii et al., 2015; Chandra et al., 2017; Šlapeta and Šlapeta, 2016; Šlapeta et al., 2018). In total, 430 new *Ctenocephalides* spp. specimens were analysed molecularly. DNA was extracted while preserving the exoskeleton as previously described (Lawrence et al., 2014). The voucher exoskeletons were clarified in KOH and dehydrated in an ethanol series and slide-mounted in Euparal (Australian Entomological Supplies, Australia) as previously described (Lawrence et al., 2014). Species confirmation and subspecies identification were performed on the mounted fleas using keys and descriptions (Hopkins and Rothschild, 1953; Segerman, 1995; Beaucournu and Menier, 1998; Ménier and Beaucournu, 1998).

2.2. Amplification of mitochondrial cytochrome c oxidase subunit I, cytochrome c oxidase subunit II and nuclear histone H3 and elongation factor 1 alpha

A subset ($n = 24/572$) including representative specimens from all collected taxa ($n = 6$ taxa; *C. f. felis, C. f. strongylus, C. orientis, C. damarensis, C. canis,* and *C. connatus*) were selected for PCR amplification using mtDNA *cox1* and *cox2*, and two nDNA markers histone H3 and EF-1α. In addition, *Echidnophaga ambulans ambulans* Olliff, 1886 (Siphonaptera: Pulicidae) was used as an outgroup. A 601 bp fragment of *cox*1 was amplified using LCO1490 (5′- GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (Folmer et al., 1994) and/or Cff-F [S0367] (5′-AGA ATT AGG TCA ACC AGG A-3′) and Cff-R [S0368] (5′-GAA GGG TCA AAG AAT GAT GT-3′) (Lawrence et al., 2014). A 727 bp fragment of *cox2* was amplified using F-Leu (5′-TCT AAT ATG GCA GAT TAG TGC-3′) and R-Lys (5′ -GAG ACC AGT ACT TGC TTT CAG TCA TC-3′) (Whiting, 2002). A 349 bp fragment of histone H3 was amplified using Hex-AF (5′- ATG GCT CGT ACC AAG CAG ACG GC -3′) and Hex-AR (5′- ATA TCC TTG GGC ATG ATG GTG AC −3′) (Zhu et al., 2015). A 933 bp of EF-1α was amplified with newly designed primers (Primer3 4.0.0): EF-1α_flea_F (5′-AAT TGA AGG CCG AAC GTG AG-3′) and EF-1α_flea_R (5′-GAT TTG CCA GTA CGA CGG TC-3′).

For all PCRs, reaction volumes of either 25 µL or 30 µL contained MyTaq Red Mix (Bioline, Australia) and approximately 1–10 ng of genomic DNA template (2 µL). All PCRs were run on a Veriti thermocycler (Life Sciences, Australia) or on a Mastercycler Personal (Eppendorf, Australia) with a negative control of PCR-grade water and a positive control of flea DNA known to amplify at conditions from previous studies (Šlapeta et al., 2011; Lawrence et al., 2014). The cycling conditions for *cox1*, EF-1α and histone H3 amplification started with 95 °C for 60 s followed by 35 cycles of 95 °C for 15 s, 55 °C for 15 s, 72 °C for 10 s, and extended for five min at 72 °C; and for *cox2* started with 95 °C for three min followed by 37 cycles of 94 °C for 30 s, 42 °C for 30 s, 72 °C for 15 s, and extended for five min at 72 °C. Products of expected sizes, verified using agarose gel electrophoresis, were bidirectionally sequenced using amplification primers (Macrogen Ltd, Seoul, Korea). All raw sequences were assembled and chromatographs visually inspected for ambiguities using CLC Main Workbench 6.9.1 (CLC bio, Denmark).

2.3. Sequence and haplotype analysis, phylogenetic diversity and ancestral state analyses

A multigene alignment was created by concatenation of *cox1, cox2*, histone H3 and EF-1α corresponding to 24 selected *Ctenocephalides* specimens and *E. a. ambulans* as an outgroup (2428 nucleotides). Using a partitioned alignment, the phylogeny was reconstructed in a Bayesian framework (BF) with either linked or unlinked parameters for General Time Reversible + γ substitution + invariant sites model (GTR + G + I) across gene partitions using MrBayes v3.2.6 (Huelsenbeck and Ronquist, 2001). Two runs with four chains of 20 million generations were performed, sampled every 200 generations and with a burn-in period set for the first 25% of trees, resulting in 75,000 total trees in the posterior distribution used to calculate posterior probability (PP). Chain mixing and convergence were visualised using the program TRACER v1.6 (Drummond and Rambaut, 2007). Nucleotide substitution models were tested using Maximum Likelihood (ML), and the GTR + G + I was selected because it had the lowest Bayesian Information Criterion (BIC) scores for the tree reconstructed using the concatenated alignment. For topological comparisons, ML and Kimura-2 (K2) distance Minimum Evolution (ME) trees with bootstrap support (1000 replicates) were also reconstructed, using MEGA 7.0.14 (Kumar et al., 2016). As a further comparison, trees for individual partitions were reconstructed using ML in MEGA7, with the best model selected using BIC scores (*cox1, cox2,* Histone H3: T92 + G, EF-1α: K2 + G) with bootstraps from 500 replicates.

A total of 584 *cox1* sequences from *Ctenocephalides* spp. were included in an alignment with *E. a. ambulans* as an outgroup. Specimens where a DNA sequence was available, but a voucher exoskeleton was not, were excluded from further analysis. Phylogeny was reconstructed in BF with GTR + G + I in MrBayes from two runs with four chains of 20 million generations, sampled every 500 generations and with a burn-in period set for the first 25% of trees, resulting in 40,000 total trees in the posterior distribution. As above, an ME tree was reconstructed from a K2 distance matrix with bootstrap (2,000 replicates) in MEGA7 for topological comparisons. The Cff-F/Cff-R amplified *cox1* region (513 bp) was used for calculating haplotype and diversity metrics on a dataset reduced to 516 sequences due to overrepresentation of Australia and New Zealand (Supplementary Table S1). Within and between taxon (or lineage) distances were calculated using K2 distance matrix. The *cox1* alignment was processed using DNAcollapser in FaBox (Villesen, 2007). The number of haplotypes (*Nh*), haplotype diversity (*h*) and nucleotide diversity (π) were calculated using DnaSP v5.10.01 (Librado and Rozas, 2009). To test whether genetic variation between groups conferred equal fitness, selective neutrality tests (Tajima's *D* and Fu's *Fs*) were also conducted in DnaSP.

We used ancestral state reconstruction of *cox1* sequences to estimate the probability that the most recent common ancestor of all sampled fleas originated in each of the sampled continents. Using an alignment of unique *cox1* sequences, the phylogeny was reconstructed in a BF with a Yule speciation prior with a Tamura-Nei + γ substitution model in BEAST v1.8.1 (Drummond and Rambaut, 2007). Two chains of 50 million generations were run, sampled every 25,000 generations and with a burn-in period of 15 million (resulting in 2,800 total trees in the posterior distribution). Chain mixing and convergence were visualised using TRACER v1.5 (Drummond and Rambaut, 2007). From the posterior distribution of 2800 trees, we calculated the core ancestor cost (CAC) to estimate the node position of each biogeographical community's most recent common ancestor (Tsirogiannis, C., Sandel, B., 2015. PhyloMeasures: fast and exact algorithms for computing phylogenetic biodiversity measures. R package version 1.1.). Larger CAC values indicate a community contains comparatively 'older' lineages of fleas. Next, for each biogeographical community we calculated the net relatedness index (NRI), a standardised phylogenetic diversity index that will be lower (i.e. more negative) in communities that are more phylogenetically diverse. To account for influences of phylogenetic uncertainty on diversity measures, the CAC and NRI indices were calculated across the full distribution of Bayesian posterior trees.

2.4. Ecological niche modelling for global Ctenocephalides populations

To identify whether ecological factors play a role in the distribution of globally ubiquitous flea species (*C. felis* and *C. canis*) and *C. felis* clusters, we employed the maximum entropy method in Maxent v3.4.1 (Phillips, S.J., Dudík, M., Schapire, R.E., 2017. Maxent software for modeling species niches and distributions (Version 3.4.1). Available at: http://biodiversityinformatics.amnh.org/open_source/maxent/. Accessed on: 18/07/2017). Maxent is a machine learning modelling approach effective in predicting species' distributions from incomplete 'presence-only' data (Phillips et al., 2006; Phillips et al., 2017). We used the geographical coordinates of each collection site for all *Ctenocephalides* specimens collected. We used two datasets, the first dataset comprised of *C. felis* and *C. canis* ($n = 483$ sequences) and a second dataset comprised of *C. felis* only ($n = 387$ sequences). Models were run using default parameters appropriate to large-scale presence-only datasets for qualitative exploratory analyses (Phillips et al., 2006; Merow et al., 2013). Predictions of habitat suitability were obtained using a global bioclimatic envelope containing 19 gridded climatic variables (Supplementary Table S3) downloaded from the WorldClim Version2 (http://worldclim.org/version2) at a grid cell resolution of 2.5 min or approximately 5 km^2 (Supplementary Table S3) (Fick and Hijmans, 2017). The bioclimatic data were converted into ESRI ASCII Raster format using QGIS (QGIS Development Team, 2017, QGIS Geographic Information System. Open Source Geospatial Foundation Project. https://www.qgis.org/). Variables relating to humidity and ambient temperature are likely to be limiting due to their importance for *Ctenocephalides* flea survival and reproduction (Silverman et al., 1981). To evaluate whether the models performed significantly better than random, 25% of each sample set was randomly selected as test data to create *P* values (significance at 0.05) relevant in ascertaining the reliability of the model. The area under curve (AUC) value for the test data on the ROC plots was used to assess the performance of each model (Fielding and Bell, 1997). The relative importance of each bioclimatic variable to the model prediction was assessed using contribution percentages and a jackknife test on training, test and AUC data.

2.5. Data accessibility

Images of representative specimens for each species, morphotype, sex and clade (https://doi.org/10.17632/tjfr3ddc52.1) and a voucher specimen list (https://doi.org/10.17632/2f3hchym9v.1) were deposited in Mendeley Data and associated analytical data are available at LabArchives (http://dx.doi.org/10.25833/2dmy-0655). Voucher specimens were deposited in the CSIRO Australian National Insect Collection (ANIC) in Canberra, Australia. All sequences were deposited in GenBank: *cox1*: MG586243-MG586672 ($n = 430$), *cox2*: MG586673-MG586732 ($n = 60$), histone H3: MG586758-MG586782 ($n = 25$), EF-1α: MG586733-MG586757 ($n = 25$); Supplementary Table S1. Sequences were also deposited in Barcode of Life Data (BOLD) Systems: CTENO001-18 to CTENO551-18, ALFLE001-14 to ALFLE024-14.

3. Results

3.1. Identification of Ctenocephalides fleas from six continents

A total of 4771 *Ctenocephalides* fleas were collected and identified across 56 countries (Table 1, Supplementary Table S1): five countries in Oceania ($n = 1446$), 16 countries in Europe ($n = 1215$), 17 countries in Asia ($n = 805$), 10 countries in Africa ($n = 571$), four countries in South America ($n = 412$) and four countries in North America ($n = 322$). An average of 85 *Ctenocephalides* fleas were collected from each country, ranging between 1 (Costa Rica) and 996 (Australia) (median = 24). Six out of the 14 known taxa for *Ctenocephalides* were identified with the aid of vouchers for 11 out of the 14 *Ctenocephalides* taxa at the Natural History Museum, London, including five holotypes and five lectotypes (Table 1, Fig. 1). Morphological identification utilised the curvature of the cephalic profile (Fig. 2) in combination with chaetotaxic features (Fig. 3), followed by further morphological discernment of sex-specific features (Fig. 4). A flowchart diagram was created detailing the identification process of *Ctenocephalides* fleas (Supplementary Fig. S1).

Fleas in the genus *Ctenocephalides* accounted for 93.1% of all fleas collected (4771/5123; Table 1, Supplementary Table S1). Only *C. felis* and *C. canis* were found across all six continents. The most common flea was *C. felis* (85.0% of total fleas collected, 4353/5123; 91.2% of total *Ctenocephalides* collected, 4353/4771) inclusive of two subspecies: *C. f. felis* (80.8%, 3856/4771) and *C. f. strongylus* (0.7%, 32/4771). This group included 29 fleas (29/4771) from Africa that could not be classified as either *C. f. felis* or *C. f. strongylus* due to intermediate cephalic morphology visible upon slide-mounting and were therefore designated as *C. f.* "transitional". The second most common was *C. canis* (4.4%, 208/4771) followed by *C. orientis* (4.1%, 194/4771), *C. connatus* (0.3%, 12/4771) and *C. damarensis* (0.1%, 4/4771). A total of 436 *C. felis* were not processed for slide-mounting and thus were not classified to subspecies level.

3.2. Multigene analysis confirms taxonomic classification of Ctenocephalides spp.

A concatenated *cox1*, *cox2*, histone H3 and EF-1α nucleotide (nt) alignment (2428 nt, 808 amino acide (aa)) was used to resolve *Ctenocephalides* phylogeny when rooted with *E. a. ambulans* (Fig. 5). In all analyses (BF, ML, ME), *C. orientis*, *C. canis* and *C. connatus* were monophyletic, together forming a sister group to *C. felis* (Fig. 5A). Two specimens, *Ctenocephalides* sp. "AL909" (morphology corresponding to *C. f. felis* – AL909-1 and *C. felis* "transitional" – AL909-2) formed a unique highly supported group (C), sister to *C. felis* (dashed line, Fig. 5A). Within *C. felis* (excluding *Ctenocephalides* sp. "AL909") we identified two lineages (A, B) supported by high ME and ML bootstrap (>90%), and high PP (>90%) for A and low PP (>55%) for B (bolded lines, Fig. 5). Within *C. felis* group A, four strongly supported (>97%) clades were resolved. No clades were consistently resolved in group B using either tree reconstruction methods. When model parameters were unlinked in BF across the concatenated partitions it provided strong PP (>90%) support

Table 1

Summary of species within the genus *Ctenocephalides* including taxonomic information, numbers of fleas analysed herein and current taxonomic assignments.

Taxa	Authority	Year	Primary host	Type locality	NHM type identifier	No# collected in this study	No# cox1 sequenced	No# multigene sequenced	Current taxonomic assignment
C. felis felis	Bouché	1835	Cats and dogs	Germany	916140	3854 + 2[e]	415 + 2[e]	9 + 2[e]	*C. f. felis*
C. felis strongylus	Jordan	1925	Cats and dogs	Kenya	916159	32 + 29[f]	32 + 29[f]	2 + 3[f]	*C. f. strongylus*
C. felis damarensis	Jordan	1936	Scrub hares (*Lepus saxatilis*) and their predators: dogs and wild canids (jackals, foxes), cats	Namibia	916156	4 [g]	4 [g]	1 [g]	*C. damarensis*
C. felis orientis	Jordan	1925	Dogs	Sri Lanka	916161	194	28	2	*C. orientis*
C. canis	Curtis	1826	Dogs and wild canids (jackals, foxes, wolves)	British Isles	916154	208	58	2	*C. canis*
C. connatus	Jordan	1925	Cape ground squirrels (*Xerus inauris*) and mongooses (Herpestidae)	South Africa	916144	12	9	3	*C. connatus*
C. rosmarus	Rothschild	1907	Dassie/Hyrax (Procaviidae)	Ethiopia	916151	0	0	0	*C. rosmarus*
C. craterus	Jordan and Rothschild	1913	Dassie/Hyrax (Procaviidae)	Kenya	916146	0	0	0	*C. craterus*
C. arabicus	Jordan	1925	Dassie/Hyrax (Procaviidae)	Yemen	916148	0	0	0	*C. arabicus*
C. crataepus	Jordan	1925	Ground squirrels (*Xerus* spp.) and hedgehogs (*Atelerix* spp.)	Kenya	916142	0	0	0	*C. crataepus*
C. paradoxuri	Wagner	1936	Civets (*Paradoxurus* spp.) and mongooses (*Herpestes vitticollis*)	Sri Lanka	916152	0	0	0	*C. paradoxuri*
C. brygooi[a]	Beaucournu	1975	Malagasy civet (*Fossa fossana*)	Madagascar	NA	0	0	0	*C. brygooi*
C. chabaudi[b]	Beaucournu and Bain	1982	Unknown[b]	Gabon	NA	0	0	0	*C. chabaudi*
C. grenieri[c]	Beaucournu and Rodhaln	1995	Dassie/rock hyrax (*Procavia capensis*)	Cameroon	NA	0	0	0	*C. grenieri*
Total						4335 + 436[d]	577 + 7[h]	24	

NHM, Natural History Museum, London, UK.

[a] Known only from type material – 7 females, 15 males.

[b] Known only by a small number of specimens collected on the bay duiker (*Cephalophus dorsalis*) (type host) and the Gambian pouched rat (*Cricetomys gambianus*).

[c] Known only from 4 specimens including type material.

[d] An additional 436 *C. felis* were not processed for slide-mounting and were not classified to subspecies level.

[e] Two specimens from Jordan (AL909-1, AL909-2) were identified as *C. f. felis* and C. f. "transitional" but later designated *Ctenocephalides* sp. "AL909" based on high genetic divergence.

[f] Total of 29 specimens from Africa had intermediate morphology between *C. f. felis* and *C. f. strongylus* and were designated *C. f.* "transitional".

[g] All specimens are male – female specimens are identical to *C. f. felis* and both species live sympatrically in South Africa, so any *C. f. damarensis* females are identified as *C. f. felis*.

[h] *C. felis* cox1 sequences for whose morphological voucher was not available (see Supplementary Table S1).

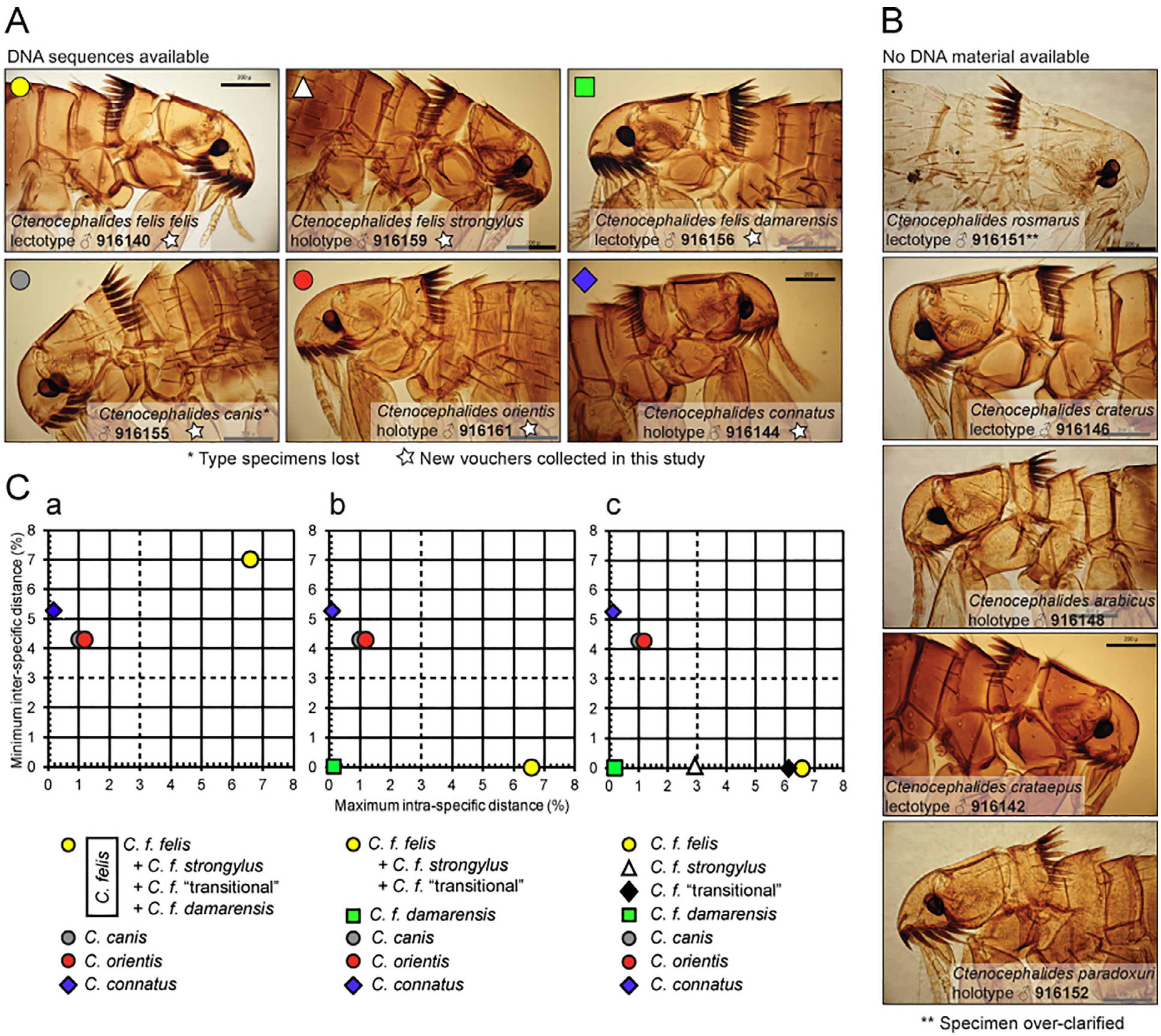

Fig. 1. *Ctenocephalides* spp. reference material. (A, B) Photographs of *Ctenocephalides* type specimens available in the Rothschild collection of fleas held at the Natural History Museum, London, UK. Stars represent taxa with new vouchers collected in this study (A), and those for which not material was not available for molecular confirmation (B). Scale bars represent 200 μm. (C) The maximum intra- and minimum inter-specific mtDNA *cox1* distances for each morphotype were calculated in three different taxonomic scenarios (a-c) based on previous literature. The first scenario (a) considers only *Ctenocephalides felis,* and does not separate *Ctenocephalides felis felis, Ctenocephalides felis strongylus, Ctenocephalides felis* "transitional" and *Ctenocephalides felis damarensis.* The second scenario (b) delineates *C. f. damarensis* as suggested by Ménier and Beaucournu (1998) based on the morphology of the male aedeagus, whilst retaining as a single category *C. f. felis*, *C. f. strongylus* and *C. felis* "transitional". The final scenario (c) treats each morphotype separately as presented in the original descriptions catalogued in Hopkins and Rothschild (1953). The evolutionary distances were calculated using the Kimura 2 (K2) parameter as recommended for closely related taxa and using *cox1* mtDNA sequences. In all graphs, a 3% threshold is enforced, dividing the charts into four separate quadrants, each representative of different species statuses (Hebert et al., 2003): top left: species concordant with current taxonomy; bottom left: species that are synonymous or have recently undergone divergence or hybridization; bottom right: probable species misidentification; top right: probable composite species, candidates for taxonomic split.

for all *Ctenocephalides* spp. as well as *Ctenocephalides* sp. "AL909" and *C. felis* group A, but it did not resolve within-group relationships (Fig. 5A). Individually, *cox1* was the most diverse marker resolving phylogeny closely resembling the concatenated tree, similarly the *cox2* tree had high bootstrap support (ML, 95%) for the *C. felis* group A (Fig. 5B). The nuclear genes resolved monophyly (ML, >90%) of *C. orientis*, *C. canis*, *C. connatus* and *C. felis* (including *Ctenocephalides* sp. "AL909") (Fig. 5B).

At the amino acid level, *cox2* contained the highest amino acid diversity with 11/228 unique variable sites, followed by the nuclear gene EF-1α with 7/264 unique sites and *cox1* with 2/200 unique sites (Fig. 5A). There were no variable amino acid residues at histone H3 marker (116 aa). The sequences of *C. canis, C. orientis, C. connatus* and *Ctenocephalides* sp. "AL909" resolved unique amino acid substitutions, but *C. damarensis* was not resolved monophyletically by any gene marker and did not exhibit a unique amino acid profile (Fig. 5A). Therefore, we revert the taxon to subspecies level, *C. f. damarensis,* and use this nomenclature henceforth. There was no discernible amino acid signal between morphologically identified *C. f. felis*, *C. f. strongylus, C. f. damarensis* and *C. f.* "transitional". The two identified *C. felis* sister groups (A, B), as well as all clades in group A were resolved using amino acid residues at *cox2* (Fig. 5A).

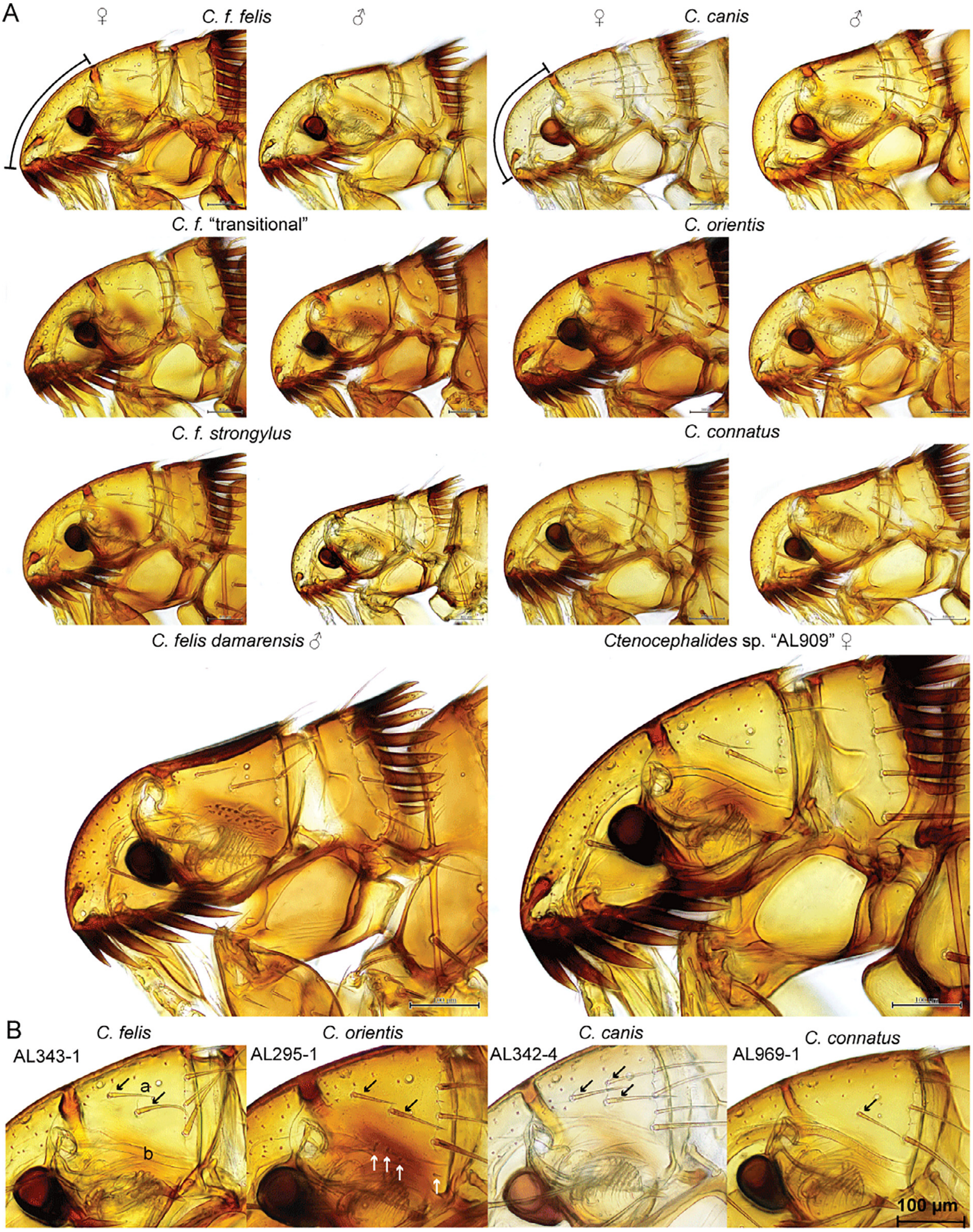

Fig. 2. Cephalic profile of six *Ctenocephalides* taxa and two morphotypes. (A) Fleas are labelled with species, subspecies or morphotype, specimen ID and sex. Female specimens of *Ctenocephalides damarensis* are indistinguishable from *Ctenocephalides felis felis* females and are therefore not represented in this figure. The *Ctenocephalides* sp. "AL909" fleas found in Jordan were morphologically identical to *C. f. felis* and no males were collected. Identification was based on genetic divergence alone. Degree of frons curvature is denoted by a curved stoppered line. Variation in frons curvature is demonstrated by the two species with the most disparate profiles: *C. f. felis*, almost straight and *Ctenocephalides canis*, heavily rounded. (B) Magnified images of the post-ocular area including the occiput (a) and post-antennal fossa (b) of four species. Black arrows denote the number of setae on the occiput of *C. f. felis* (two setae), *C. orientis* (two setae), *C. canis* (three setae) and *C. connatus* (one seta). White arrows denote the presence of micro-setae behind the antennal fossa of *C. orientis* females only. All scales are 100 µm.

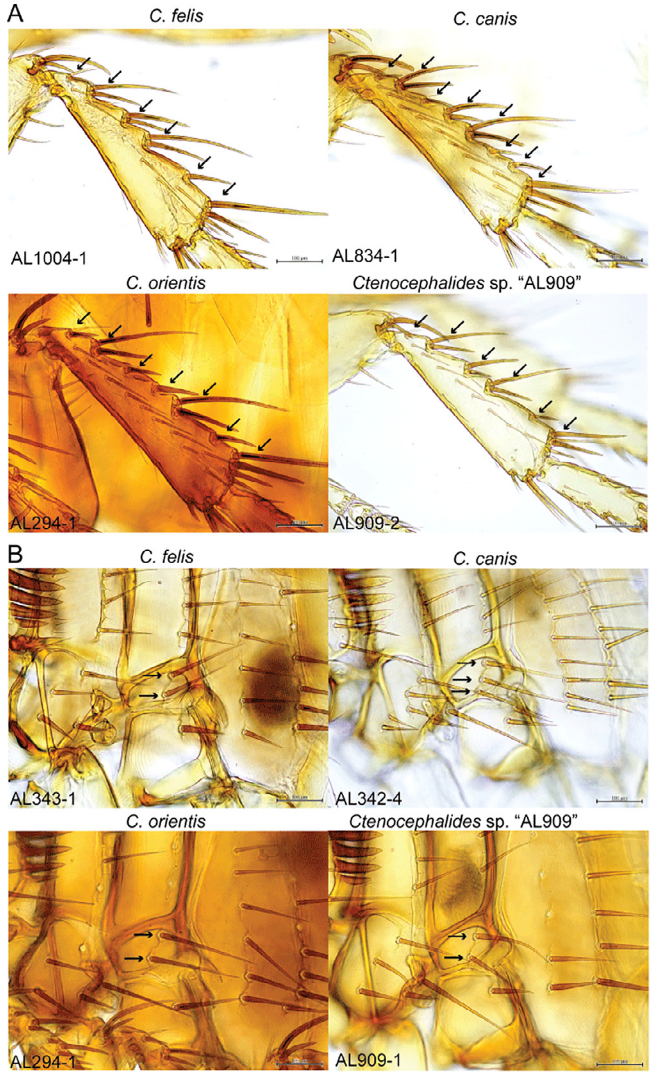

Fig. 3. Morphology of hind tibia (A) and lateral metanotal area (LMA) (B) for *Ctenocephalides felis*, *Ctenocephalides canis*, *Ctenocephalides orientis* and *Ctenocephalides* sp. "AL909". Fleas are labelled with species or subspecies and specimen ID. (A) Arrows denote the number of setae bearing notches present on the dorsoposterior margin of the hind tibia of *Ctenocephalides felis felis* (six), *C. canis* (eight), *C. orientis* (seven), and *Ctenocephalides* sp. "AL909" (six). (B) Arrows denote the number of setae on the LMA with *C. f. felis*, *C. orientis* and *Ctenocephalides* sp. "AL909" all bearing two setae and *C. canis* bearing three. All specimens are female. All scales are 100 μm.

3.3. Incongruence between morphology and genetic signature for *C. felis* subspecies

The *cox1* gene region provided the highest nucleotide diversity (Fig. 5B) and thus *cox1* sequences were PCR amplified from 430 morphologically identified *Ctenocephalides* spp. vouchers (Supplementary Table S1). Phylogenetic analysis of the *cox1* DNA alignment demonstrated monophyly for *C. felis* as a species group with both high PP (100%) and bootstrap (98%) support (Fig. 6). However, the morphologically identified *C. felis* subspecies (i.e. *C. f. felis*, *C. f. strongylus*, *C. f. damarensis*) and the *C. f.* "transitional" morphotype were not conserved monophyletically by any gene marker (Fig. 5). *Ctenocephalides* sp. "AL909" formed a strongly supported monophyletic sister lineage (C) to *C. felis* (Fig. 6) (PP: 100%, bootstrap: 96%). In addition, *cox1* sequences of *C. canis*, *C. orientis* and *C. connatus* were unambiguously resolved into monophyletic groups (PP: 100%, bootstrap: >99%). Within *C. felis*, *cox1* sequences were not clustered according to their morphological traits, i.e. subspecies identity. Both sister lineages A and B within *C. felis* included specimens morphologically classified as *C. f. felis*, *C. f. strongylus*, *C. f. damarensis* and the *C. f.* "transitional" morphotype. The phylogenetic trees demonstrated the existence of eight clades within *C. felis* (Clades 1–8), however the PP or bootstrap support was low for most clades (Fig. 5B and Fig. 6).

To investigate whether *cox1* correctly identifies *Ctenocephalides* spp. and subspecies, the maximum intra- and minimum interspecific *cox1* distances (%) were calculated with morphologically confirmed *Ctenocephalides* spp. and subspecies (Fig. 1) The calculated distances were plotted on a grid split into four quadrants using an arbitrary 3% *cox1* barcode distance threshold (Hebert et al., 2003) (Fig. 1C). The top left quadrant was occupied by *C. canis, C. orientis* and *C. connatus,* implying support of taxonomic species status by *cox1* (Fig. 1Ca-c). The top right quadrant denoting probable composite species was occupied by *C. felis*; subspecies were lumped together (Fig. 1Ca). To assess support for *C. f. damarensis*, the specimens were treated individually whilst the remaining *C. felis* subspecies were grouped (Fig. 1Cb). Separation of *C. f. damarensis* resulted in <3% inter- and intraspecific distance denoting recent species divergence, hybridization or synonymy. Regardless of whether *C. f. felis, C. f. strongylus* and *C. f.* "transitional" were grouped together or treated individually, the intraspecific distance was high whilst the interspecific distance was effectively zero, demonstrating that the *cox1* signal does not correspond to morphological traits (Fig. 1Cb-c).

3.4. High genetic diversity of *C. felis* fleas indicates out-of-Africa origins

Out of a total of 90 unique *cox1* haplotypes, 55 belonged to *C. felis* specimens (Fig. 7A). Across the *Ctenocephalides* spp. sampled, *C. felis* exhibited the highest genetic diversity (Table 2). The largest number of *cox1* haplotypes for *C. felis* was found in Africa ($n = 24$), followed by Asia and Oceania (Table 2). Three *C. felis cox1* haplotypes (h1, h2 and h17) represented 46% of the total sequences with h1 (*C. felis*) being the most common and widespread haplotype ($n = 145$, 28% of total sequences) (Fig. 7B). Haplotypes h1, h2 and h17 were distributed globally, but with continental predominance to Europe, Asia and the Americas, respectively (Fig. 7B). A total of 54 *cox1* haplotypes (10% of total sequences) were singletons. Phylogeny of *cox1* haplotypes in general agreed with Fig. 6, except for Clade 5 that was paraphyletic (Fig. 7).

A number of morphologically defined *C. felis* subspecies shared a single *cox1* haplotype in the African region (h6, h17, h21, h22, h27). Morphological inconsistencies across the *C. felis* haplotype primarily consisted of variations in the cephalic profile for both sexes and in the aedeagal morphology (Ménier and Beaucournu, 1998) and tarsal chaetotaxy in male fleas (Beaucournu and Menier, 1998) (Table 3 and Table 4). Moreover, a single haplotype h6 was found in the Central African Republic, Seychelles and Georgia USA representing *C. f. strongylus*, *C. f.* "transitional" and *C. f. felis*, respectively (Fig. 7). The subspecies *C. f. felis* and *C. f. strongylus* exhibit a tubus interior that may be toothed in four out of five specimens (Table 3). Our data show that the rate is much higher with the tubus interior toothed in half of the specimens (Table 4).

Africa displayed the highest genetic diversity and the highest probability for being the geographical origin of the ancestral root of *Ctenocephalides* spp. at 20.1% compared with 15.0–16.8% for all other continents (Table 2, Supplementary Fig. S2). The African *C. felis* community had the highest CAC values (95% Confidence Interval (CI): 61.44, 70.35; indices for all other continents were <14.78) and most negative NRI values (95% CI: −9.36, −7.56; indices for all other continents were >−6.96), denoting a more

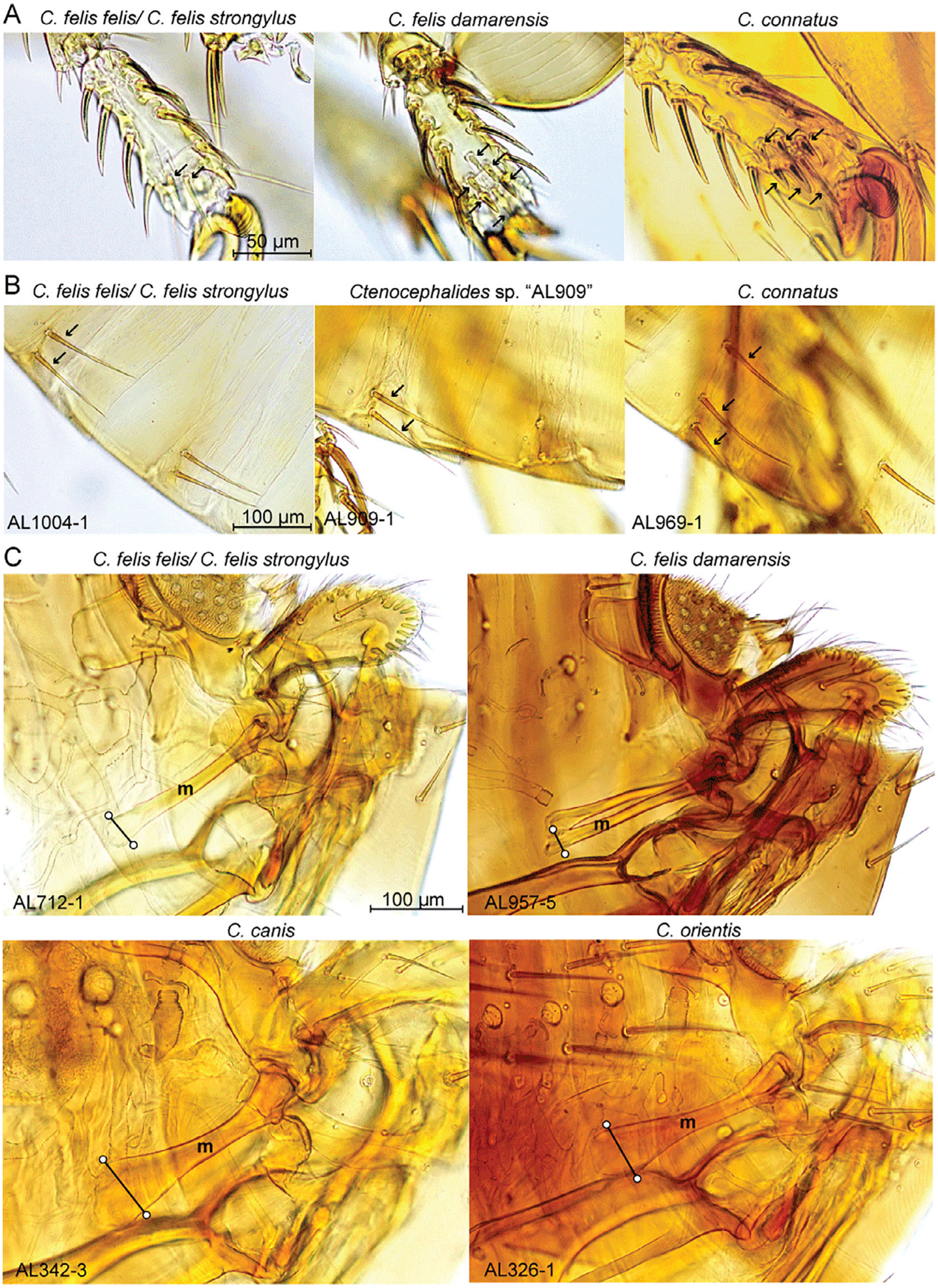

Fig. 4. Sex-specific morphology of *Ctenocephalides* fleas. (A) Plantar surface of fore tarsi V of male fleas. Number of spiniform bristles on the plantar surface is denoted by black arrows: two for *Ctenocephalides felis felis*, six for *Ctenocephalides damarensis* and *Ctenocephalides connatus*. (B) Ventral sternites of female fleas. Number of sub-ventral setae on sternites (3 – 6) are indicated with black arrows for *C. f. felis* (two), *Ctenocephalides* sp. "AL909" (two) and *C. connatus* (three). (C) Manubrium (m) of male fleas. *Ctenocephalides f. felis* and *C. damarensis* possess a manubrium with a constricted apex, whilst for *C. canis* and *C. orientis* the apex of the manubrium is dilated, denoted by the stoppered line. Fleas are labelled with species or subspecies and specimen ID. Scale for (A) is 50 μm and scale for (B) and (C) is 100 μm.

ancient and phylogenetically diverse flea community compared with other continents. Within *C. felis*, a large portion of fleas show a more recent European ancestral line. Similarly, *C. canis* and *C. orientis* show recent European and Asian ancestry, respectively (Supplementary Fig. S2). There was no significant difference between "ancient" (Africa, Europe, Oceania and Asia) and "recently colonised" (North and South America) regions ($P > 0.1$) using an analysis of molecular variance (AMOVA) in an attempt to determine if patterns of genetic diversity reflected patterns of phylogenetic diversity.

Using mtDNA *cox1* sequences we calculated neutrality values to evaluate population structure and determine whether *Ctenocephalides* populations are evolving non-randomly (Table 2). Only *C. canis* had statistically significant negative neutrality values,

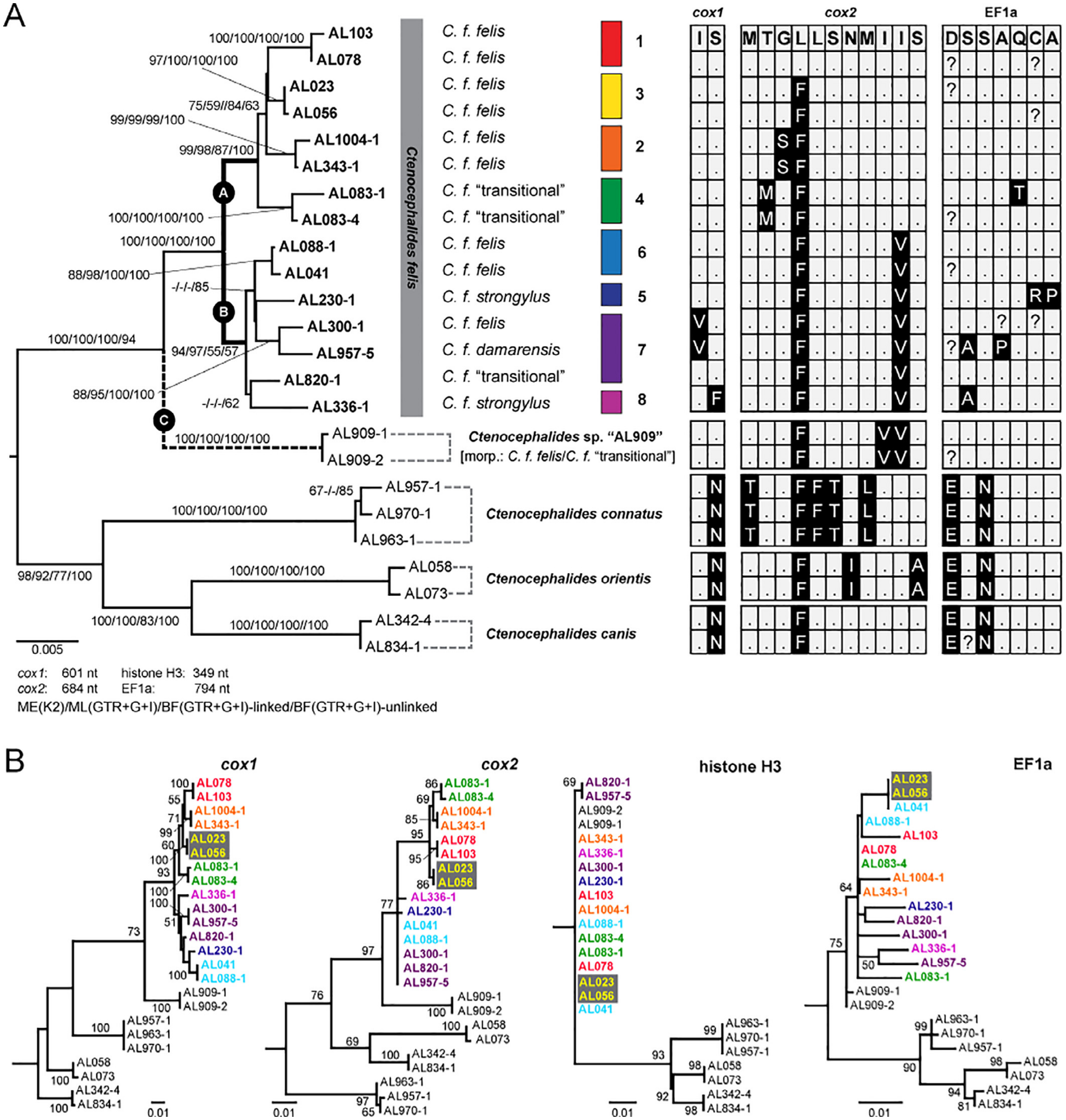

Fig. 5. Phylogenetic analysis of the fleas in the genus *Ctenocephalides* inferred from mitochondrial and nuclear DNA markers. The evolutionary history was inferred using the Minimum Evolution (ME), Maximum Likelihood (ML) and Bayesian framework (BF) with either linked or unlinked parameters (A). A multigene alignment was created by concatenation of *cox1*, *cox2*, histone H3 and EF-1α corresponding to 24 selected *Ctenocephalides* specimens and *Echidnophaga ambulans ambulans* as an outgroup (2428 nucleotides). Substitution models with the lowest Bayesian Information Criterion (BIC) scores were selected using model selection (MEGA 7.0.14). The GTR + G + I model was used for the ML tree. The ME tree was reconstructed using K2 distance matrix. The BF tree was created using either linked GTR + G + I parameters (BF-linked) or unlinked GTR + G + I parameters (BF-linked) across the gene partitions. Branch support is shown, including bootstrap support (%) using 2000 replicates (ME) and 1000 replicates (ML), followed by posterior probability (PP) based on 75,000 trees (BF). Specimens are identified by their voucher identifier followed by species or subspecies/morphotype. At least one representative of each morphotype was selected. Sequence alignment of the amino acid variable sites for each clade and species is shown on the right. Residues identical to top sequence are displayed as dots (.). Question marks (?) denote codons containing variable nucleotides. Trees for individual partitions were reconstructed using ML using substitution models with the lowest BIC (*cox1*, *cox2*, Histone H3: T92 + G, EF-1α: K2 + G) with bootstraps from 500 replicates (B). Branch values lower than 50% were discarded. Trees were rooted using *Echidnophaga ambulans ambulans* (Siphonaptera: Pulicidae) – not shown.

suggesting recent population expansion (Fig. 7A). Within *C. felis*, fleas from North and South America had statistically significant population structure with positive neutrality values due to the presence of a small number of distinct haplotypes and a low number of singletons, suggesting multiple introductions and sustained isolation (Fig. 7A).

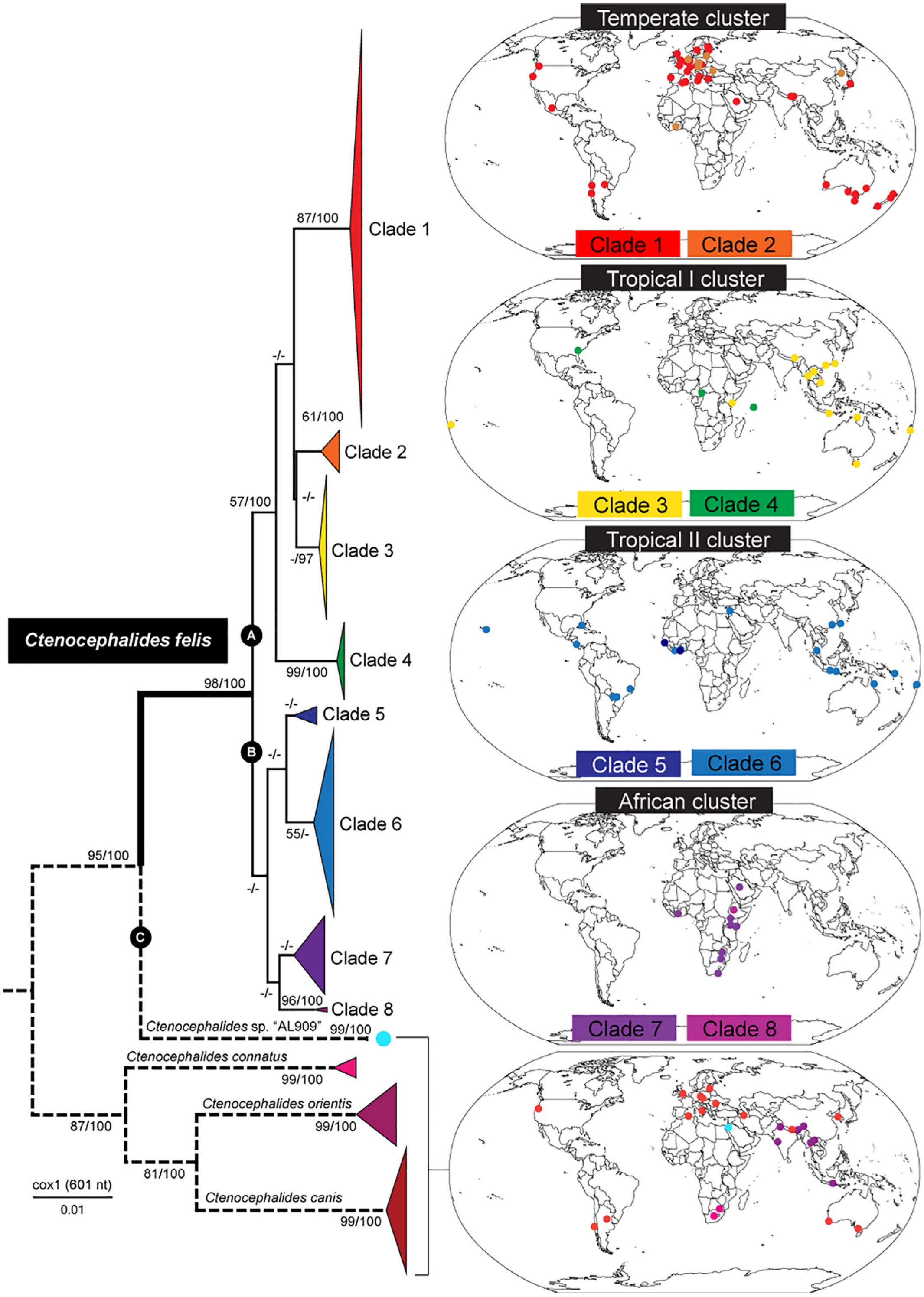

Fig. 6. Phylogenetic reconstruction of *Ctenocephalides* spp. at *cox1*. Sequences from fleas ($n = 520$) from 56 countries across six continents were rooted with *Echidnophaga ambulans ambulans* sequences ($n = 4$, not shown). The evolutionary history was inferred using Minimum Evolution (ME) with distances computed using the Kimura 2 (K2) method and Bayesian framework (BF) with GTR + G + I parameters. Branch support is shown, including bootstrap support (%) using 2000 replicates (ME) followed by posterior probability (PP) based on 40,000 trees (BF). Branch values lower than 50% were discarded. Clade identity is denoted by colour with collection sites for each sequenced flea plotted on a world map. Maps are divided by lineage for *C. felis* or per individual species.

3.5. Niche modelling on a global scale reveals ecologically discrete C. felis clusters and temperate distribution of C. canis

Discrete geographical clusters were observed when the coordinates of *C. felis* collection sites were plotted on a world map and associated with their *cox1* Clades 1–8 (Fig. 6); arbitrarily labelled: 'Temperate' (Clade 1 and Clade 2), 'Tropical I' (Clade 3 and Clade 4), 'Tropical II' (Clade 5 and Clade 6) and 'African' (Clade 7 and Clade 8) clusters (Fig. 6 and Fig. 8). The distribution pattern of *C. canis* was climatically restricted to the temperate zone in both the northern

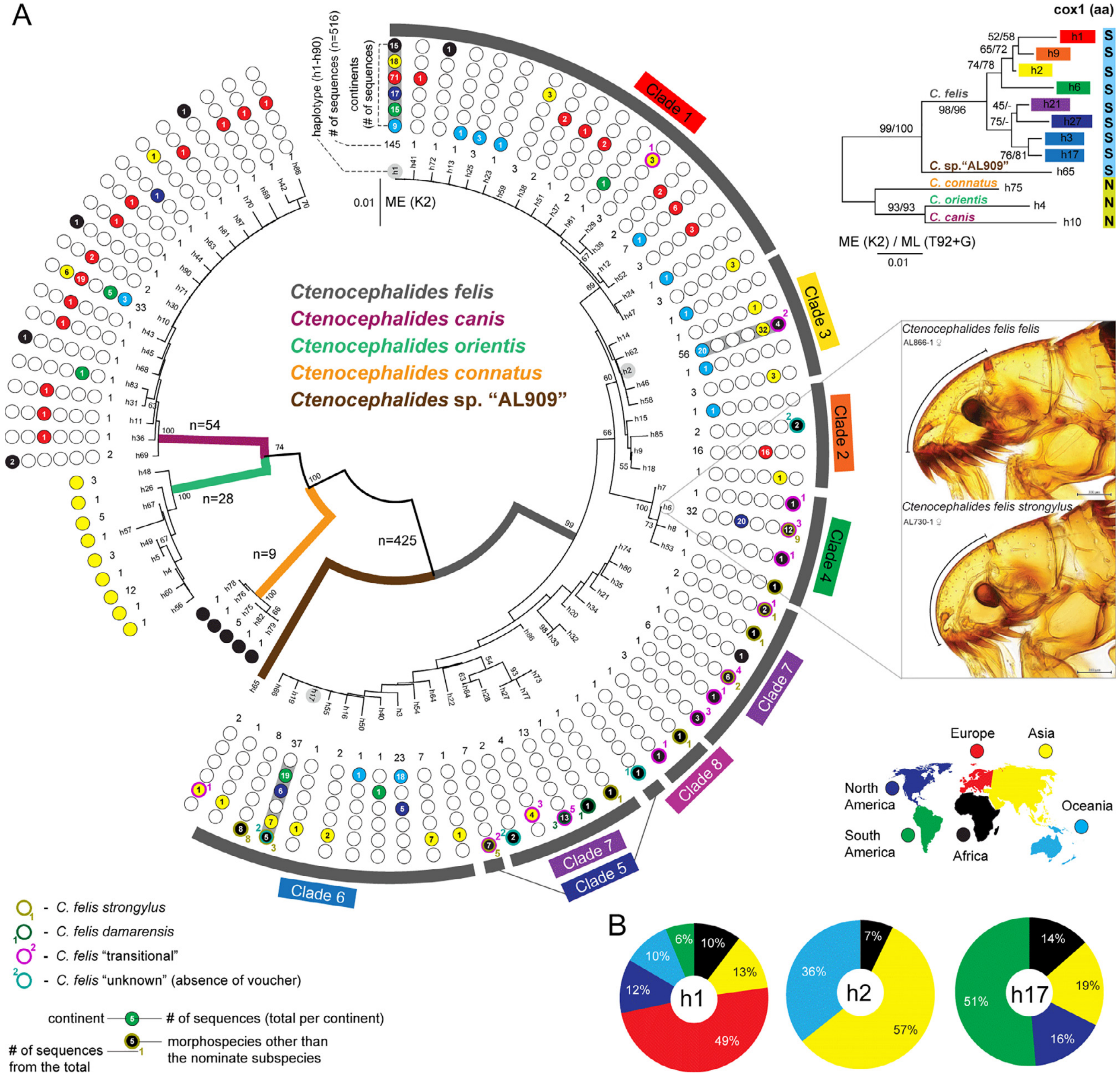

Fig. 7. Phylogenetic relationships and geographic distribution of haplotypes across *Ctenocephalides* morphologies. (A) Phylogenetic reconstruction of all unique *cox1* haplotypes recovered in the study, inferred using Minimum Evolution (ME) and the K2 distance matrix. Bootstrap support values from 1000 replicates are shown at the branches, values lower than 50% were discarded. The terminal node is labelled with the haplotype ID, followed by number of sequences found for each haplotype. The radiating circles show the continent (colour-coded) and the number of sequences found per continent. The three most abundant haplotypes are shaded grey. For *Ctenocephalides felis*, subspecies or morphotypes that deviate from the nominal subspecies *Ctenocephalides felis felis* are annotated. Species lineages are denoted with colour-coded branches. (B) Phylogenetic relationships and amino acid translations for the most common haplotypes of each *C. felis cox1* clade except Clade 8 (only two fleas, different haplotypes). Two methods were used to infer evolutionary history: ME with K2 distance model and Maximum Likelihood (ML) with the T92 + γ model. Bootstrap values (1000 replicates) for both runs are shown and those lower than 50% were discarded. Amino acid translations for the mtDNA sequences show unique sites differentiating *C. felis* from all other species. (C) Morphology was not conserved across identical haplotypes. For example, haplotype h6 contains fleas identified as *C. f. felis* and *Ctenocephalides felis strongylus*, respectively. These subspecies are differentiated by the degree of curvature of the frons, as indicated by the stoppered line. The scales represent 100 μm. (D) The three most globally abundant haplotypes (h1, h2 and h17) are shown with the proportion of members found in each continent.

(not found lower than 27° N) and southern hemispheres (no further north than 31°S) (Fig. 6). The species *C. connatus* was restricted to South Africa. The oriental dog flea (*C. orientis*) was restricted to Asia and located throughout the tropical and northern subtropical zones (Fig. 6).

We tested the probability of the geographical clusters being constrained by bioclimatic factors (Fig. 8). Models predicting localities for *C. felis* and *C. canis* as well as *C. felis* (Temperate, Tropical I and Tropical II) displayed high predictive power (AUC > 0.9). All distribution models predicted test localities significantly better than random ($P < 0.001$). The *C. felis* model displayed a highly cosmopolitan distribution whilst the model for *C. canis* demonstrated a temperately constricted distribution with virtually no suitable habitats highlighted along the equatorial belt, except at high altitudes such as the Himalayas (Fig. 8B). A Jackknife test showed that the *C. felis* Temperate cluster and *C. canis* shared the same bioclimatic predictors (Supplementary Fig. 3A, E). Variables that related to cold temperatures such as minimum temperature of the coldest

Table 2
Summary of genetic diversity and demographic statistics for *Ctenocephalides* spp., *Ctenocephalides felis* clades and continental populations of *C. felis.*

				Genetic diversity statistics		Tests of selective neutrality					
	n	*Hn*	*S*	*h* (∓SD)	π(∓SD)	Tajima's *D*	Stat. sig.	Fu and Li's *D*[a]	Stat. sig.	Fu and Li's *F*[a]	Stat. sig.
By species											
C. felis	424	55	49	0.846 (0.014)	0.01562 (3.9×10^{-5})	0.23	No	−0.51	No	−0.20	No
C. canis	54	20	25	0.630 (0.078)	0.00222 (4.4×10^{-4})	−2.56	Yes [c]	−4.59	Yes[b]	−4.60	Yes[b]
C. orientis	28	9	12	0.783 (0.065)	0.00351 (7.1×10^{-4})	−1.38	No	−1.75	No	−1.91	No
C. connatus	9	5	7	0.722 (0.159)	0.00509 (1.4×10^{-3})	0.06	No	−0.05	No	−0.03	No
Ctenocephalides sp. "AL909"	2	1	0	NA	NA	NA	NA	NA	NA	NA	NA
By clade											
Clade 1	179	17	15	0.342 (0.046)	0.00087 (1.4×10^{-4})	−2.13	Yes[a]	−2.63	Yes[a]	−2.93	Yes[a]
Clade 2	19	3	3	0.292 (0.127)	0.00098 (4.6×10^{-4})	−1.13	No	−0.11	No	−0.44	No
Clade 3	64	6	5	0.207 (0.067)	0.00042 (1.4×10^{-4})	−1.84	Yes[a]	−2.93	Yes[a]	−3.03	Yes[a]
Clade 4	35	4	3	0.166 (0.084)	0.00033 (1.7×10^{-4})	−1.73	No	−2.79	Yes[a]	−2.88	Yes[a]
Clade 5	8	2	3	0.250 (0.180)	0.00146 (1.1×10^{-3})	−1.45	No	−1.57	No	−1.69	No
Clade 6	82	10	12	0.780 (0.036)	0.00371 (2.8×10^{-4})	−0.58	No	−1.54	No	−1.43	No
Clade 7	35	11	13	0.825 (0.05)	0.00629 (3.8×10^{-4})	0.07	No	−0.81	No	−0.62	No
Clade 8	2	2	0	NA	NA	NA	NA	NA	NA	NA	NA
By continent											
Africa	91	24	36	0.918 (0.012)	0.01800 (5.3×10^{-4})	0.95	No	0.05	No	0.48	No
Asia	85	14	25	0.793 (0.033)	0.01224 (8.6×10^{-4})	0.79	No	0.38	No	0.64	No
Europe	104	9	13	0.51 (0.055)	0.00379 (5.7×10^{-4})	−0.59	No	0.31	No	−0.02	No
North America	48	4	21	0.689 (0.036)	0.01703 (8.9×10^{-4})	2.73	Yes[b]	1.70	Yes[b]	2.44	Yes[b]
Oceania	66	13	24	0.789 (0.026)	0.01331 (8.7×10^{-4})	1.11	No	−0.66	No	−0.02	No
South America	30	4	16	0.524 (0.074)	0.01281 (1.7×10^{-3})	2.13	Yes[a]	0.81	No	1.44	No

n, number of sequences = number of fleas.
Hn, number of haplotypes.
S, number of variable sites.
h (∓SD), haplotype diversity plus/minus S.D.
π(∓SD), nucleotide diversity plus/minus S.D.

[a] $P < 0.05$.
[b] $P < 0.02$.
[c] $P < 0.001$.

Table 3
Distinguishing features of male *Ctenocephalides felis* subspecies and *Ctenocephalides felis damarensis* as per previous literature.

Species	Number of SPB[a] on fore tarsi	Phallosome structures	
		TI[b] toothed	Hamulus length: width
C. f. felis/strongylus	2	4 out of 5 are toothed	≈2.5× longer than wide
C. f. damarensis	4–6	Small tooth	approximately equal

[a] Spiniform plantar bristles.
[b] Tubus interior.

month (BIO06) and mean temperature of the coldest quarter (BIO11) contributed the most information to the model and were the best predictors of distribution when used in isolation from all other variables (Supplementary Fig. S3A, E). Conversely, variables relating to precipitation (annual precipitation – BIO12 and precipitation of the warmest quarter – BIO18) were the best predictors of habitat distribution for the two Tropical clusters of *C. felis* (Supplementary Fig. S3B-C). The most significant contributors to the *C. felis* species distribution model were a mixture of factors relating to cold temperature such as mean temperature of the coldest quarter (BIO11) and precipitation, such as precipitation in the coldest quarter (BIO19) (Supplementary Fig. S3D).

4. Discussion

We demonstrate 'out-of-Africa' origins of *C. felis* and elucidate its taxonomy using a global collection of *Ctenocephalides* spp. from 56 countries. Our data provide empirical evidence of the ubiquity of the cat flea on a global scale, where cat and dog domestication and subsequent human-mediated migration has led to a worldwide distribution of *C. felis*. We challenge the dogma that *C. felis* specimens collected in regional studies from different countries around the world are morphologically, phylogenetically and biologically identical.

We demonstrate that *C. felis* originated in Africa because (i) the continent holds the highest *C. felis* genetic diversity and (ii) *C. felis* from Africa possess the highest CAC values, denoting a more ancient population compared with other continents. Our analysis demonstrates an African common ancestor for all *Ctenocephalides* taxa collected in our study, therefore aligning with the proposed African origin of the Pulicidae (Traub, 1985; Medvedev, 1998).

The evolution of pulicid fleas, including *C. felis*, likely occurred simultaneously with their carnivorous hosts, thereby providing the means for early dispersal (Zhu et al., 2015). The African wildcat (*F. s. lybica*) had a distribution range that extended throughout Africa and the Near East, supporting our hypothesis of an African origin of *C. felis* (Driscoll et al., 2007). After Africa, the most ancient flea communities were found in Asia, Europe, then Oceania while the Americas held the most recently evolved cat flea populations. These flea populations correspond to the origins of domestication and the translocation of cats and dogs into new geographical locations by humans (Milham and Thompson, 1976; Savolainen et al., 2004; Oskarsson et al., 2011).

The earliest evidence of cat domestication was discovered in China and the group of now extinct ancestral wolves that gave rise to the gray wolf (*Canis lupus*) and the domestic dog (*Canis lupus familiaris*) originated in Europe (Thalmann et al., 2013; Hu et al., 2014). Dog domestication is thought to have occurred over two independent events, one in western Eurasia and another in eastern Eurasia approximately 15,000 years ago; they were the first domesticated animal to undergo human-mediated dispersal to all continents (Oskarsson et al., 2011; Larson et al., 2012; Frantz et al., 2016). These early domestication events are reflected in the older, genetically diverse flea populations of Asia and Europe.

Table 4
Table of conflicting diagnostic features of all male *Ctenocephalides felis* and *Ctenocephalides felis damarensis* fleas collected from Africa.

Specimen information					Number of SPB[a] on fore tarsi	Phallosome structures	
Morphotype	Clade ID	Haplotype ID	Specimen ID	Country		TI[b] toothed?	Hamulus ratio length:width
C. f. felis	Clade 1	h1	AL796-4	Algeria	2	No	1.6
C. f. felis	Clade 1	h1	AL796-5	Algeria	2	No	1.5
C. f. felis	Clade 1	h1	AL798-1	Algeria	2	No	1.6
C. f. felis	Clade 1	h1	AL913-1	Algeria	2	No	2.3
C. f. felis	Clade 1	h1	AL917-1	Algeria	2	Yes	1.8
C. f. felis	Clade 7	h27	AL300-1	South Africa	2	Yes	2.2
C. f. “transitional”	Clade 3	h2	AL340-1	Kenya	2	Yes	1.3
C. f. “transitional”	Clade 3	h2	AL341-1	Kenya	2	No	1.8
C. f. “transitional”	Clade 7	h21	AL336-4	Kenya	2	Yes	2.3
C. f. “transitional”	Clade 7	h21	AL820-3	Zimbabwe	2	Yes	2.3
C. f. strongylus	Clade 5	h22	AL222-1	Guinea Bissau	2	No	1.8
C. f. strongylus	Clade 5	h22	AL229-1	Guinea Bissau	2	No	2.7
C. f. strongylus	Clade 4	h6	AL725-1	CAR[c]	2	Yes	2.0
C. f. strongylus	Clade 4	h6	AL728-1	CAR[c]	2	Yes	2.0
C. f. strongylus	Clade 6	h19	AL1113-2	Liberia	2	Yes	1.4
C. f. strongylus	Clade 6	h19	AL1113-3	Liberia	2	Yes	1.8
C. f. strongylus	Clade 6	h19	AL1113-5	Liberia	NA[d]	Yes	1.8
C. f. strongylus	Clade 6	h17	AL1113-10	Liberia	2	Yes	2.0
C. f. strongylus	Clade 7	h73	AL937-1	South Africa	2	No	1.8
C. f. strongylus	Clade 7	h80	AL965-1	Kenya	2	Yes	1.3
C. f. damarensis	Clade 7	h27	AL946-2	South Africa	5	Yes	1.1
C. f. damarensis	Clade 7	h77	AL957-4	South Africa	6	Yes	1.3
C. f. damarensis	Clade 7	h27	AL957-5	South Africa	6	Yes	1.7
C. f. damarensis	Clade 7	h27	AL957-6	South Africa	5	Yes	1.6

[a] Spiniform plantar bristles.
[b] Tubus interior.
[c] Central African Republic.
[d] Unable to determine due to specimen damage.

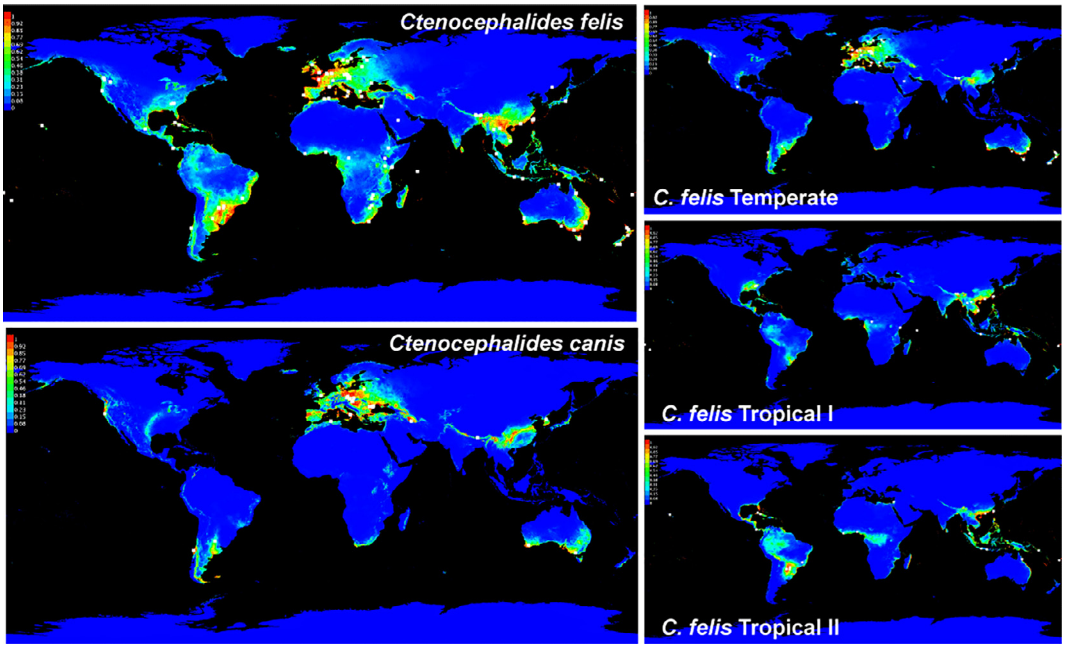

Fig. 8. Predicted global geographic distribution of suitable habitat zones for *Ctenocephalides felis* and *Ctenocephalides canis*. Three subgroups of *C. felis* (see Fig. 6) distributions were modelled – Temperate, Tropical I and Tropical II. The prediction is based on *Ctenocephalides* flea collection sites and 19 variables forming a bioclimatic envelope. A colour scale is used to show the predicted probability of habitat suitability with red indicating high probability that the bioclimatic conditions in the area are suitable for the species or lineage, green indicating conditions that are similar to those where the specimens are found and light blue indicating low probability of suitable conditions. White markers represent the presence of a single flea.

Given the close phylogenetic relationship of *C. canis* and *C. orientis*, and their dual affinity for canine hosts, the European and Asian distribution and ancestry of *C. canis* and *C. orientis*, respectively, could be a reflection of these domestication events. Evidence suggests that humans began migrating out of Africa as early as 120,000 years ago but did not reach the Americas until 15,000 years ago, possibly explaining the more recently evolved community of cat fleas in the Americas, characterised by low genetic diversity (Goebel et al., 2008; Stringer, 2011, 2016; Groucutt et al., 2015).

Maritime exploration and trade from the 15th to the 19th century allowed the dispersal of cats and probably dogs, providing an opportunity for rapid flea dispersal and admixture between continental populations (Koch et al., 2015, 2016). The near-boundless global distributions of the genetically homogeneous *C. felis* Clade 1 and of *C. canis* could be a reflection of European exploration and colonisation. Our data show that *C. canis* was not evolving neutrally and negative selective neutrality values suggest rapid recent global expansion. This mirrored the population structure of Clade 1 of *C. felis,* a similarly temperately distributed population. Fleas (*C. felis*) belonging to Clade 1 are the most globally ubiquitous and these fleas are now dominant in Australia and New Zealand (Šlapeta et al., 2011; Chandra et al., 2017). This genetic homogeneity within globally distributed populations likely denotes rapid expansion and consequent founder effects in each area as a result of modern human movement and globalisation (Avise, 2009).

Africa is the only continent that holds all eight clades of *C. felis* and recent human-mediated dispersal of *C. felis* could, in part, explain the genetic homogeneity found among continental populations that are geographically separated. This includes the *C. felis* in Clade 4 (haplotype h6, 32/35 fleas) from the Central African Republic, the Seychelles and Georgia, USA. Both the Seychelles and southern USA were involved in the transatlantic slave trade from the 16th to the 19th century with human cargo being sourced from West Africa, including the region now called the Central African Republic; although the Seychelles was more heavily influenced by traders coming from eastern Africa (Avery, 2017; Lovejoy, 2011). These historic trade routes affected the dispersal of domestic animals and their parasites throughout the world and likely resulted in the introduction of flea populations into novel environments from Africa.

Similar to all ectoparasites with 'off-host' life stages, *Ctenocephalides* flea growth and reproduction is highly dependent upon environmental conditions and climatic factors such as air temperature and precipitation or humidity (Silverman et al., 1981; Dryden and Rust, 1994; Silverman and Rust, 1983; Dryden and Rust, 1994). The results of our analysis showed that, as a species, *C. felis* demonstrates high levels of ecological plasticity with a broad spectrum of suitable bioclimatic regions highlighted across multiple climatic zones. Conversely, no *C. canis* were found between the two Tropics and the species displayed strict temperate habitat distribution.

Analysis of the relative importance of environmental variables used in a species distribution model allows insight into which environmental factors represent the most significant drivers for species distribution (Booth et al., 2014). Our results showed that variables relating to cooler temperatures were best for predicting the distribution of *C. canis* fleas. This corroborates laboratory bioassays that reported narrower temperature ranges for optimal growth in *C. canis* (Baker and Elharam, 1992). Cat fleas (*C. f. strongylus*) from equatorial Africa including the Ivory Coast, Guinea Bissau and Liberia, fell within the Tropical I cluster and showed ecological predilection for warmer, more humid habitats. This aligns with experimental data demonstrating that the subspecies *C. f. strongylus* completes its lifecycle slower at temperatures lower than 27 °C when compared with *C. f. felis*; the optimal temperature for development being 29 °C compared with 27 °C for *C. f. felis* (Yao et al., 2006). Our work supports in vitro biological observations, despite the dataset possibly being subject to spatial bias due to our opportunistic sampling methods (Lobo Jorge et al., 2007; Fourcade et al., 2014). The relative contribution of bioclimatic values, however, is known to be relatively heuristic due to the nature of the algorithm and should be interpreted with care when environmental variables are highly correlated (Phillips et al, 2006; Phillips et al, 2017).

Our results confirm that the cat flea, *C. felis*, is the most common flea infesting domestic cats and dogs around the world. The taxonomic classification of the global subspecies has not been systematically addressed using molecular data. Using a multigene approach coupled with comprehensive morphological analysis, we resolve the taxonomic ambiguity of *C. felis* as a species and demonstrate the presence of three subspecies: the cosmopolitan *C. f. felis*, recovered from every continent, and two subspecies restricted to the African region: *C. f. strongylus* and *C. f. damarensis*.

The subspecies *C. f. damarensis* was raised to full species level during the 1990 s based on morphological investigations and historical observations of host preferences (De Meillon et al., 1961; Louw and Horak, 1995; Ménier and Beaucournu, 1998). We show little to no genetic distance between *C. f. damarensis* morphotypes and other *C. felis* morphotypes from Africa. Similarly, despite the morphological differences between *C. f. felis* and *C. f. strongylus* morphotypes, many of these specimens shared the same or very similar genetic identity. Previous studies have attempted to synonymise *C. f. strongylus* with *C. f. felis* due to difficulties in differentiating the two in Africa, where the subspecies reside sympatrically (Ménier and Beaucournu, 1998; Vobis et al., 2004). Our work demonstrates that despite the presence of discernible morphological differences between *C. f. felis*, *C. f. strongylus* and *C. f. damarensis*, the subspecies lack genetic monophyly.

Most taxomomists agree that the definition of a subspecies dictates that the group of taxa must be recognisable by at least one feature or set of features, and that the taxa are geographically defined in some way (Patten, 2015; Wallin et al., 2017). Unlike in full species, reproductive isolation and reciprocal monophyly is not a prerequisite for the definition of a subspecies (Mayr, 1942; Patten, 2015). As such, when geographical barriers separating the subspecies are overcome, hybridisation can occur, leading to genetic homogeneity (Mayr, 1942; Patten, 2015). Due to the cosmopolitan distribution of the nominated subspecies *C. f. felis*, including distribution throughout Africa, hybridisation between this subspecies and both African subspecies is highly probable, particularly since they can share the same hosts. The presence of 'intermediate' specimens in Africa classified here as *C. f.* "transitional" are possibly evidence of existing hybridisation between *C. f. felis* and *C. f. strongylus*.

The mitochondrial gene markers provided the highest phylogenetic resolution compared with the nuclear genes for *Ctenocephalides* fleas. Since the nuclear genome evolves at a slower rate compared with the mitogenome, the diversity seen in *cox1* and *cox2* may represent more recent divergences and thus utilisation of all four genes reveals the most comprehensive phylogenetic picture (Avise, 2009). Using the multigene approach, *C. connatus, C. canis* and *C. orientis* retained monophyly regardless of the gene marker, supporting full species status for these taxa. The species *C. orientis* was observed to have a strong canine host preference and a close phylogenetic relationship with *C. canis*. The species is also known to infest small ruminants such as goats and sheep throughout Asia, supporting the retention of *C. orientis* as a full species (Ashwini et al., 2017). The fact that there are very few historical cases of *C. orientis* collected from cats further corroborates this theory (Hopkins and Rothschild, 1953; Beaucournu and Menier, 1998). Collection and genetic profiling of the remaining species in genus *Ctenocephalides,* particularly those in Africa, is pertinent and pressing in order to resolve the taxonomy and phylogeny of the genus as a whole. This remains a monumental task, given that many of these species are very rare with uncertain host affinities (Beaucournu and Menier, 1998).

In summary, we confirm the full species status of *C. orientis* — originally described as an Asian subspecies of *C. felis* — which together with robust morphological separation and discerning host preferences, formed a discrete monophyletic unit. Despite the taxonomic challenge presented by the intermediate specimens in Africa, the *C. felis* subspecies nonetheless exhibit conserved identifiable features; therefore, we reinstate and confirm the subspecies status of the following within *C. felis*: cosmopolitan *C. f. felis*, *C. f. strongylus* in Africa and Arabian Peninsula, and *C. f. damarensis* in South Africa.

With a global collection of *Ctenocephalides* spp., we demonstrated African origins of the genus and subsequent human-mediated dispersal of *C. felis* as a result of cat and dog domestication. Through synanthropic host hitchhiking, the cat flea has successfully achieved global dominance. The success of *C. felis* can be attributed to high phylogenetic diversity and discrete climatic clusters, allowing ecological plasticity of the species as a whole. Using an integrated morphological and molecular approach, we taxonomically resolved *C. felis*, demonstrating the presence of three subspecies: cosmopolitan *C. f. felis* and two African subspecies *C. f. strongylus* and *C. f. damarensis,* whilst confirming the full species status of *C. canis*, *C. orientis* and *C. connatus.* Our study demonstrates the ancestral origin of the most common flea on Earth and exposes the drivers behind its global dispersal, thereby defining the factors associated with this parasite's success.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2019.01.001. These data include Google maps of the most important areas described in this article.

References

Ashwini, M.S., Puttalakshmamma, G.C., Mamatha, G.S., Chandranaik, B.M., Thimmareddy, P.M., Placid, E., Jalali, S.K., Venkatshan, T., 2017. Studies on morphology and molecular characterization of oriental cat flea infesting small ruminants by barcoding. J. Entomol. Zool. Stud. 5, 301–305.

Avery, S., 2017. Seychelles Political History and Governance. Lulu Press Inc, Morrisville, NC.

Avise, J.C., 2009. Phylogeography: retrospect and prospect. J. Biogeogr. 36, 3–15.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., Saunders, N.C., 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18, 489–522.

Baker, K.P., Elharam, S., 1992. The biology of *Ctenocephalides canis* in Ireland. Vet. Parasitol. 45, 141–146.

Beaucournu, J.C., Menier, K., 1998. Le genre *Ctenocephalides* Stiles et Collins, 1930 (Siphonaptera, Pulicidae). Parasite 5, 3–16.

Booth, T.H., Nix, H.A., Busby, J.R., Hutchinson, M.F., 2014. bioclim: the first species distribution modelling package, its early applications and relevance to most current MaxEnt studies. Divers. Distrib. 20, 1–9.

Chandra, S., Forsyth, M., Lawrence, A.L., Emery, D., Šlapeta, J., 2017. Cat fleas (*Ctenocephalides felis*) from cats and dogs in New Zealand: molecular characterisation, presence of *Rickettsia felis* and *Bartonella clarridgeiae* and comparison with Australia. Vet. Parasitol. 234, 25–30.

Clark, N.J., Seddon, J.M., Šlapeta, J., Wells, K., 2018. Parasite spread at the domestic animal - wildlife interface: anthropogenic habitat use, phylogeny and body mass drive risk of cat and dog flea (*Ctenocephalides* spp.) infestation in wild mammals. Parasit. Vectors 11, 8.

De Meillon, B., Davis, D.H.S., Hardy, F., 1961. The Siphonaptera (excluding Ischnopsyllidae). Plague in Southern Africa. Govt. Printer. of South Africa, Pretoria, Republic.

Driscoll, C.A., Menotti-Raymond, M., Roca, A.L., Hupe, K., Johnson, W.E., Geffen, E., Harley, E.H., Delibes, M., Pontier, D., Kitchener, A.C., Yamaguchi, N., Brien, S.J., Macdonald, D.W., 2007. The Near Eastern origin of cat domestication. Science 317, 519.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Dryden, M.W., Rust, M.K., 1994. The cat flea: biology, ecology and control. Vet. Parasitol. 52, 1–19.

Dunnet, G., Mardon, D., 1974. A monograph of Australian fleas (Siphonaptera). Aust. J. Zool. Suppl. Ser. 22, 1–273.

Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37, 4302–4315.

Fielding, A.H., Bell, J.F., 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. Environ. Conserv. 24, 38–49.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Fourcade, Y., Engler, J.O., Rodder, D., Secondi, J., 2014. Mapping species distributions with MAXENT using a geographically biased sample of presence data: a performance assessment of methods for correcting sampling bias. PLoS One 9, e97122.

Frantz, L.A.F., Mullin, V.E., Pionnier-Capitan, M., Lebrasseur, O., Ollivier, M., Perri, A., Linderholm, A., Mattiangeli, V., Teasdale, M.D., Dimopoulos, E.A., Tresset, A., Duffraisse, M., McCormick, F., Bartosiewicz, L., Gál, E., Nyerges, É.A., Sablin, M.V., Bréhard, S., Mashkour, M., Bălăşescu, A., Gillet, B., Hughes, S., Chassaing, O., Hitte, C., Vigne, J.-D., Dobney, K., Hänni, C., Bradley, D.G., Larson, G., 2016. Genomic and archaeological evidence suggest a dual origin of domestic dogs. Science 352, 1228.

Goebel, T., Waters, M.R., Rourke, D.H., 2008. The late Pleistocene dispersal of modern humans in the Americas. Science 319, 1497.

Groucutt, H.S., Petraglia, M.D., Bailey, G., Scerri, E.M.L., Parton, A., Clark-Balzan, L., Jennings, R.P., Lewis, L., Blinkhorn, J., Drake, N.A., 2015. Rethinking the dispersal of *Homo sapiens* out of Africa. Evol. Anthropol. 24, 149–164.

Hebert, P.D., Cywinska, A., Ball, S.L., deWaard, J.R., 2003. Biological identifications through DNA barcodes. Proc. Biol. Sci. 270, 313–321.

Hii, S.-F., Lawrence, A.L., Cuttell, L., Tynas, R., Abd Rani, P., Šlapeta, J., Traub, R., 2015. Evidence for a specific host-endosymbiont relationship between '*Rickettsia* sp. genotype RF2125' and *Ctenocephalides felis orientis* infesting dogs in India. Parasit. Vectors 8, 169.

Hopkins, G.H.E., Rothschild, M., 1953. Volume 1: Tungidae and Pulicidae. An illustrated catalogue of the Rothschild collection of fleas (Siphonaptera) in the British Museum (Natural History): with keys and short descriptions for the identification of families, genera, species and subspecies. The Trustees of the British Museum, London.

Hu, Y., Hu, S., Wang, W., Wu, X., Marshall, F.B., Chen, X., Hou, L., Wang, C., 2014. Earliest evidence for commensal processes of cat domestication. Proc. Natl. Acad. Sci. 111, 116–120.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Koch, K., Algar, D., Schwenk, K., 2016. Feral cat globetrotters: genetic traces of historical human-mediated dispersal. Ecol. Evol. 6, 5321–5332.

Koch, K., Algar, D., Searle, J.B., Pfenninger, M., Schwenk, K., 2015. A voyage to Terra Australis: human-mediated dispersal of cats. BMC Evol. Biol. 15, 262.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Larson, G., Karlsson, E.K., Perri, A., Webster, M.T., Ho, S.Y.W., Peters, J., Stahl, P.W., Piper, P.J., Lingaas, F., Fredholm, M., 2012. Rethinking dog domestication by integrating genetics, archeology, and biogeography. Proc. Natl. Acad. Sci. 109, 8878–8883.

Lawrence, A.L., Brown, G.K., Peters, B., Spielman, D.S., Morin-Adeline, V., Šlapeta, J., 2014. High phylogenetic diversity of the cat flea (*Ctenocephalides felis*) at two mitochondrial DNA markers. Med. Vet. Entomol. 28, 330–336.

Lawrence, A.L., Hii, S.-F., Jirsová, D., Panáková, L., Ionică, A.M., Gilchrist, K., Modrý, D., Mihalca, A.D., Webb, C.E., Traub, R.J., Šlapeta, J., 2015a. Integrated morphological and molecular identification of cat fleas (*Ctenocephalides felis*) and dog fleas (*Ctenocephalides canis*) vectoring *Rickettsia felis* in central Europe. Vet. Parasitol. 210, 215–223.

Lawrence, A.L., Hii, S.F., Chong, R., Webb, C.E., Traub, R., Brown, G., Šlapeta, J., 2015b. Evaluation of the bacterial microbiome of two flea species using different DNA-isolation techniques provides insights into flea host ecology. FEMS Microbiol. Ecol., 91

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.

Lobo Jorge, M., Jiménez-Valverde, A., Real, R., 2007. AUC: a misleading measure of the performance of predictive distribution models. Global Ecol. Biogeogr. 17, 145–151.

Louw, J.P., Horak, M.L., 1995. Fleas, lice and mites on scrub hares (*Lepus saxatilis*) in northern and eastern Transvaal and in KwaZulu-Natal, South Africa. Onderstepoort. J. Vet. Res. 62, 133–137.

Lovejoy, P.E., 2011. Transformations in Slavery: A History of Slavery in Africa. Cambridge University Press, Cambridge, UK.

Ma, C., Yang, P., Jiang, F., Chapuis, M.P., Shali, Y., Sword, G.A., Kang, L.E., 2012. Mitochondrial genomes reveal the global phylogeography and dispersal routes of the migratory locust. Mol. Ecol. 21, 4344–4358.

Mayr, E., 1942. Systematics and the Origin of Species, from the Viewpoint of a Zoologist. Harvard University Press, Cambridge, MA.

Medvedev, S.G., 1998. Classification of fleas (order Siphonaptera) and its theoretical foundations. Entomol. Rev. 78, 1080–1093.

Ménier, K., Beaucournu, J.-C., 1998. Taxonomic study of the genus *Ctenocephalides* Stiles & Collins, 1930 (Insecta: Siphonaptera: Pulicidae) by using aedeagus characters. J. Med. Entomol. 35, 883–890.

Merow, C., Smith, M.J., Silander, J.A., 2013. A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. Ecography 36, 1058–1069.

Milham, P., Thompson, P., 1976. Relative antiquity of human occupation and extinct fauna at Madura Cave, southeastern Western Australia. Aust. J. Anthropol. 10, 175–180.

Oskarsson, M.C.R., Klütsch, C.F.C., Boonyaprakob, U., Wilton, A., Tanabe, Y., Savolainen, P., 2011. Mitochondrial DNA data indicate an introduction through mainland Southeast Asia for Australian dingoes and Polynesian domestic dogs. Proc. R. Soc. Lond. B. Biol. Sci. rspb20111395.

Patten, M.A., 2015. Subspecies and the philosophy of science. The Auk 132, 481–485.

Phillips, S.J., Anderson, R.P., Dudík, M., Schapire, R.E., Blair, M.E., 2017. Opening the black box: an open-source release of Maxent. Ecography 40, 887–893.

Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190, 231–259.

Rust, K.M., 2017. The biology and ecology of cat fleas and advancements in their pest management: A review. Insects 8.

Rust, M.K., Dryden, M.W., 1997. The biology, ecology, and management of the cat flea. Annu. Rev. Entomol. 42, 451–473.

Savolainen, P., Leitner, T., Wilton, A.N., Matisoo-Smith, E., Lundeberg, J., 2004. A detailed picture of the origin of the Australian dingo, obtained from the study of mitochondrial DNA. Proc. Natl. Acad. Sci. USA 101, 12387–12390.

Savolainen, P., Zhang, Y.-P., Luo, J., Lundeberg, J., Leitner, T., 2002. Genetic evidence for an East Asian origin of domestic dogs. Science 298, 1610–1613.

Segerman, J., 1995. Siphonaptera of Southern Africa: Handbook for the Identification of Fleas. South African Institute for Medical Research, Johannesburg, South Africa.

Silverman, J., Rust, M.K., 1983. Some abiotic factors affecting the survival of the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). Environ. Entomol. 12, 490–495.

Silverman, J., Rust, M.K., Reierson, D.A., 1981. Influence of temperature and humidity on survival and development of the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). J. Med. Entomol. 18, 78–83.

Šlapeta, J., King, J., McDonell, D., Malik, R., Homer, D., Hannan, P., Emery, D., 2011. The cat flea (*Ctenocephalides f. felis*) is the dominant flea on domestic dogs and cats in Australian veterinary practices. Vet. Parasitol. 180, 383–388.

Šlapeta, J., Lawrence, A., Reichel, M.P., 2018. Cat fleas (*Ctenocephalides felis*) carrying *Rickettsia felis* and *Bartonella* species in Hong Kong. Parasitol. Int. 67, 209–212.

Šlapeta, Š., Šlapeta, J., 2016. Molecular identity of cat fleas (*Ctenocephalides felis*) from cats in Georgia, USA carrying *Bartonella clarridgeiae*, *Bartonella henselae* and *Rickettsia* sp. RF2125. Vet. Parasitol. Reg. Stud. Rep. 3, 36–40.

Stringer, C., 2011. The Origin of Our Species. Penguin, UK.

Stringer, C., 2016. Human migration: Climate and the peopling of the world. Nature 538, 49–50.

Thalmann, O., Shapiro, B., Cui, P., Schuenemann, V.J., Sawyer, S.K., Greenfield, D.L., Germonpré, M.B., Sablin, M.V., López-Giráldez, F., Domingo-Roura, X., Napierala, H., Uerpmann, H.P., Loponte, D.M., Acosta, A.A., Giemsch, L., Schmitz, R.W., Worthington, B., Buikstra, J.E., Druzhkova, A., Graphodatsky, A.S., Ovodov, N.D., Wahlberg, N., Freedman, A.H., Schweizer, R.M., Koepfli, K.P., Leonard, J.A., Meyer, M., Krause, J., Pääbo, S., Green, R.E., Wayne, R.K., 2013. Complete mitochondrial genomes of ancient canids suggest a European origin of domestic dogs. Science 342, 871.

Traub, R., 1985. Coevolution of Fleas and Mammals. In: Lim, K.C. (Ed.), Coevolution of Parasitic Arthropods and Mammals. Wiley, New York, pp. 295–437.

Villesen, P., 2007. FaBox: an online toolbox for fasta sequences. Mol. Ecol. Notes 7, 965–968.

Vobis, M., D'Haese, J., Mehlhorn, H., Mencke, N., Blagburn, B.L., Bond, R., Denholm, I., Dryden, M.W., Payne, P., Rust, M.K., Schroeder, I., Vaughn, M.B., Bledsoe, D., 2004. Molecular phylogeny of isolates of *Ctenocephalides felis* and related species based on analysis of ITS1, ITS2 and mitochondrial 16S rDNA sequences and random binding primers. Parasitol. Res. 94, 219–226.

Wallin, H., Kvamme, T., Bergsten, J., 2017. To be or not to be a subspecies: description of *Saperda populnea lapponica* ssp. n. (Coleoptera, Cerambycidae) developing in downy willow (*Salix lapponum* L.). ZooKeys 691, 103–148.

Weinstein, S.B., Kuris, A.M., 2016. Independent Origins of Parasitism in Animalia. Biol, Lett, p. 12.

Whiting, M.F., 2002. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. Zool. Scr. 31, 93–104.

Windsor, D.A., 1998. Controversies in parasitology, most of the species on earth are parasites. Int. J. Parasitol. 28, 1939–1941.

Yao, K.P., Ngoran, K.E., Franc, M., 2006. Étude de quelques paramètres écologiques de *Ctenocephalides felis strongylus* (Jordan, 1925) (Siphonaptera: Pulicidae). Parasite 13, 159–164.

Zhu, Q., Hastriter, M.W., Whiting, M.F., Dittmar, K., 2015. Fleas (Siphonaptera) are Cretaceous, and evolved with Theria. Mol. Phylogen. Evol. 90, 129–139.