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Cat fleas (*Ctenocephalides felis*) carrying *Rickettsia felis* and *Bartonella* species in Hong Kong

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ABSTRACT

Fleas are commonly recorded on stray as well as domestic dogs and cats in Hong Kong. Fleas can be a major cause of pruritus in dogs and cats and also vectors of potentially zoonotic bacteria in the genera *Rickettsia* and *Bartonella*. Morphological examination of 174 fleas from dogs and cats living in Hong Kong revealed only cat fleas (*Ctenocephalides felis*). Cytochrome c oxidase subunit 1 gene (*cox1*) genotyping of 20 randomly selected specimens, revealed three *cox1* haplotypes (HK-h1 to HK-h3). The most common haplotype was HK-h1 with 17 specimens (17/20, 85%). HK-h1 was identical to *cox1* sequences of fleas in Thailand and Fiji. HK-h1 and HK-h2 form a distinct cat flea *cox1* clade previously recognized as the Clade 3. HK-h3 forms a new Clade 6. A multiplex *Bartonella* and *Rickettsia* real-time PCR of DNA from 20 *C. felis* found *Bartonella* and *Rickettsia* DNA in three (15%) and ten (50%) *C. felis*, respectively. DNA sequencing confirmed the presence of *R. felis*, *B. clarridgeiae* and *Bartonella henselae*. This is the first reported study of that kind in Hong Kong, and further work is required to expand the survey of companion animals in the geographical region. The sampling of fleas on domestic cats and dogs in Hong Kong revealed them to be exclusively infested by the cat flea and to be harbouring pathogens of zoonotic potential.

1. Introduction

Fleas (Siphonaptera) are not only a nuisance to small animals, causing irritation and allergies [1], but are also vectors of a variety of pathogens, such as *Bartonella*, *Rickettsia*, *Ehrlichia* and haemoplasma species in cats and dogs, and wildlife [2–7]. In addition, some members of the genus *Bartonella* and *Rickettsia* have a zoonotic capacity [8–16].

In Australia and New Zealand [17,18], the predominant species affecting, both cats and dogs is the cat flea, *Ctenocephalides felis* (Bouche, 1835), but this might vary in different regions of the world [19]. Reports from Asia seem to predominantly focus on the fleas of rodents, and their potential as reservoirs of the Plague (*Yersinia pestis*) [20–22], although there are scant reports on the ectoparasites (and fleas) of domestic animals [23,24]. There do not appear to be any scientific reports from Hong Kong on the fleas of domestic cats and dogs, possibly a reflection of its lack of a local centre of veterinary research, but anecdotally they are known to be a major irritant to the small animal populations. Besides cat fleas, a dog flea (*Ctenocephalides canis*), Oriental cat flea (*Ctenocephalides orientis*) and human flea (*Pulex irritans*) are also frequently reported on dogs and cats worldwide or in the case of the Oriental cat flea in South East Asia [19,25,26]. Whether they harbor pathogens is unknown, but historically Hong Kong is famously known for the discovery of *Yersinia pestis* as the causal organism of the plague [27].

The present work was aimed at providing evidence of the identity of fleas on dog and cats in Hong Kong, and whether these carry any pathogens of human and/or veterinary significance.

2. Materials and methods

2.1. Flea specimens

Fleas were collected from free-roaming cats (n = 8) and dogs (n = 9) in Hong Kong (Table 1, Fig. 1). Fleas collected from individual animals were stored in 70–100% ethanol in the freezer (− 20 °C) until processed.

2.2. Morphological identification

Fleas were identified to the species level using a stereo microscope

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Table 1
Location, collection date and species of the host ((free-roaming) cat or dog), identity, sex and total number of fleas (n = 174) collected from 17 cats and dogs in Hong Kong, SAR China.

ID	Flea species	Host	Fleas			Location	Collection date
			No#	Female	Male		
CAT1	*C. felis*	Cat	11	5	6	1	2 September 2015
CAT2	*C. felis*	Cat	12	10	2	1	9 September 2015
CAT3	*C. felis*	Cat	12	10	2	1	10 September 2015
CAT4	*C. felis*	Cat	11	8	3	1	14 September 2015
CAT5	*C. felis*	Cat	8	3	5	1	22 September 2015
CAT6	*C. felis*	Cat	10	6	4	1	23 September 2015
CAT7	*C. felis*	Cat	9	8	1	1	29 September 2015
CAT8	*C. felis*	Cat	12	9	3	2	6 November 2015
DOG0	*C. felis*	Dog	8	6	2	1	July/August 2015
DOG1	*C. felis*	Dog	11	9	2	1	2 September 2015
DOG2	*C. felis*	Dog	8	7	1	1	7 September 2015
DOG3	*C. felis*	Dog	10	10	0	2	6 November 2015
DOG4	*C. felis*	Dog	9	9	0	2	6 November 2015
DOG5	*C. felis*	Dog	10	4	6	2	11 November 2015
DOG6	*C. felis*	Dog	11	11	0	2	6 November 2015
DOG7	*C. felis*	Dog	11	11	0	2	6 November 2015
DOG8	*C. felis*	Dog	11	10	1	2	17 October 2015
Total			174	136	38		

Location 1 (Latitude 22.3964 N; Longitude 114.1095 E).
Location 2 (Latitude 22.4950 N; Longitude 114.0696 E).

(5–20 × objectives, BX41, Olympus, Australia). Flea species, sex and number of flea(s) per animal were recorded with the aid of available keys and descriptions [28].

2.3. DNA extraction of fleas

Twenty *Ctenocephalides felis* (*C. felis*) were randomly selected for genotyping, targeting the cytochrome *c* oxidase subunit I (*cox1*) gene. Total flea genomic DNA was extracted using Isolate II Genomic DNA Kit (BioLine, Australia) and eluted in 100 µL of elution buffer as previously described [29]. Voucher specimens were cleared and mounted in Euparal (Ento Supplies, Australia).

2.4. Amplification of the mitochondrial encoded cytochrome c oxidase subunit I

A 601-nt 5′ fragment of the *cox1* gene was amplified in a polymerase chain reaction (PCR) using a generic invertebrate amplification forward primer, LCO1490 (5′–GGT CAA CAA ATC ATA AAG ATA TTG G–3′) and a reverse primer designed in a previous study Cff-R [S0368] (5′–GAA GGG TCA AAG AAT GAT GT–3′) [29,30]. PCR amplification included MyTaqTM Red Mix (BioLine, Australia) as described previously [29]. PCR products were sequenced (Macrogen Ltd., Seoul, South Korea) deposited to GenBank (*cox1*: KY417905 – KY417924). DNA sequences were assembled using CLC Main Workbench 6.8.1 (CLC bio, Qiagen, Denmark). Phylogenetic analysis of *Ctenocephalides* spp. *cox1* representatives obtained from GenBank was determined using MEGA 7.0 [31].

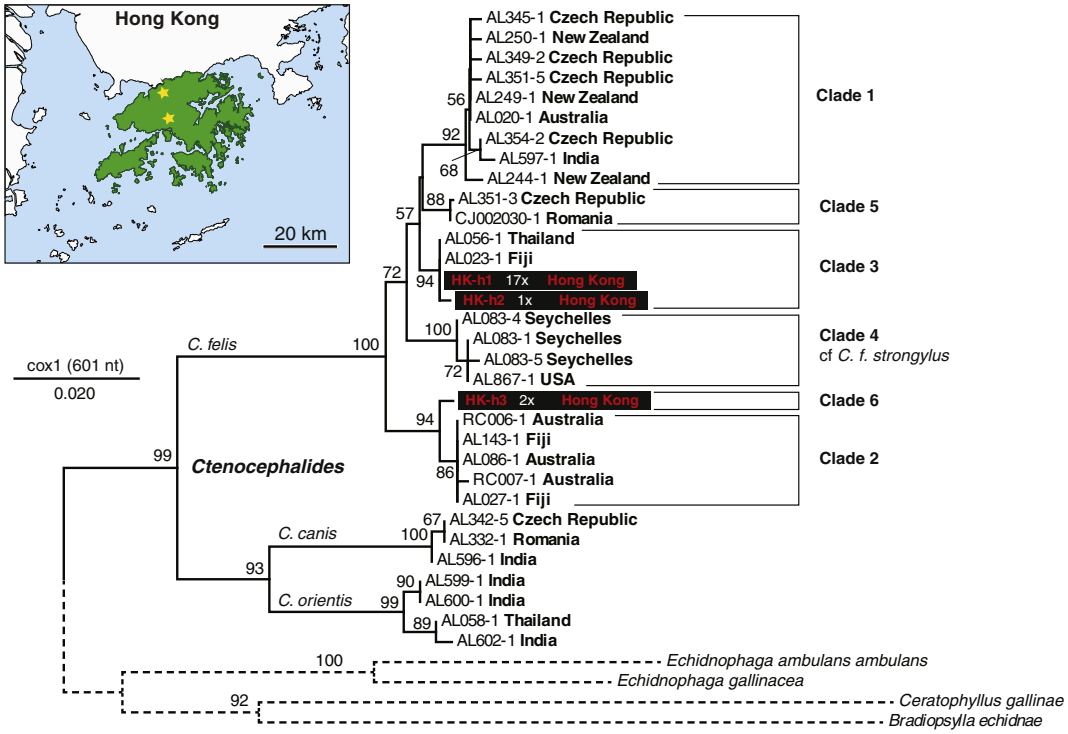

Fig. 1. Phylogenetic analysis of cat fleas (*Ctenocephalides felis*) from Hong Kong. The evolutionary history of the *cox1* was inferred using the Minimum Evolution (ME) method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (> 50%). The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 41 nucleotide sequences of *cox1* mtDNA. The analysis involved 36 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 601 positions in the final alignment. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Outgroup is indicated by dotted branches. Evolutionary analyses were conducted in MEGA7. The inset shows the Hong Kong map and locations (stars) from where the animals with fleas were collected from.