Genetic characterization of flea-derived *Bartonella* species from native animals in Australia suggests host-parasite co-evolution

Gunn Kaewmongkol, Sarawan Kaewmongkol, Linda M. McInnes, Halina Burmej, Mark D. Bennett, Peter J. Adams, Una Ryan, Peter J. Irwin, Stanley G. Fenwick

PII: S1567-1348(11)00276-0
DOI: 10.1016/j.meegid.2011.07.021
Reference: MEEGID 1075

To appear in: *Infection, Genetics and Evolution*

Received Date: 21 June 2011
Revised Date: 26 July 2011
Accepted Date: 28 July 2011

Please cite this article as: Kaewmongkol, G., Kaewmongkol, S., McInnes, L.M., Burmej, H., Bennett, M.D., Adams, P.J., Ryan, U., Irwin, P.J., Fenwick, S.G., Genetic characterization of flea-derived *Bartonella* species from native animals in Australia suggests host-parasite co-evolution, *Infection, Genetics and Evolution* (2011), doi: 10.1016/j.meegid.2011.07.021

Genetic characterization of flea-derived *Bartonella* species from native animals in Australia suggests host-parasite co-evolution

Gunn Kaewmongkol

School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch 6150, Western Australia, Australia

Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

Sarawan Kaewmongkol

Faculty of Veterinary Technology, Kasetsart University, Bangkok, Thailand

Center for Agricultural Biotechnology (AG-BIO/PEDRO-CHE), Thailand

Linda M. McInnes

School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch 6150, Western Australia, Australia

Halina Burmej

School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch 6150, Western Australia, Australia

Mark D. Bennett

School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch 6150, Western Australia, Australia

Peter J. Adams

School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch 6150, Western Australia, Australia

Una Ryan

School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch 6150, Western Australia, Australia

Peter J. Irwin

School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch 6150, Western Australia, Australia

Stanley G. Fenwick

School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch 6150, Western Australia, Australia

Address for Correspondence

Gunn Kaewmongkol, School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Perth, Western Australia, Australia, 6150

Email address: G.Kaewmongkol@murdoch.edu.au

Abstract

Fleas are important arthropod vectors for a variety of diseases in veterinary and human medicine, and bacteria belonging to the genus *Bartonella* are among the organisms most commonly transmitted by these ectoparasites. Recently, a number of novel *Bartonella* species and novel species candidates have been reported in marsupial fleas in Australia. In the present study the genetic diversity of marsupial fleas was investigated; ten species of fleas were collected from 7 different marsupial and placental mammal hosts in Western Australia including woylies (*Bettongia penicillata*), western barred bandicoots (*Perameles bougainville*), mardos (*Antechinus flavipes*), bush rats (*Rattus fuscipes*), red foxes (*Vulpes vulpes*), feral cats (*Felis catus*) and rabbits (*Oryctolagus cuniculus*). PCR and sequence analysis of the cytochrome oxidase subunit I (*COI*) and the 18S rRNA genes from these fleas was performed. Concatenated phylogenetic analysis of the *COI* and 18S rRNA genes revealed a close genetic relationship between marsupial fleas, with *Pygiopsylla hilli* from woylies, *Pygiopsylla tunneyi* from western barred bandicoots and *Acanthopsylla jordani*

from mardos, forming a separate cluster from fleas collected from the placental mammals in the same geographical area. The clustering of *Bartonella* species with their marsupial flea hosts suggests co-evolution of marsupial hosts, marsupial fleas and *Bartonella* species in Australia.

Keywords: Co-evolution, *Bartonella* species, fleas, native animals, Australia

1. Introduction

Fleas (order Siphonaptera, meaning wingless-tube) are blood-sucking insects that mostly infest mammals and some avian species. They are hosts for a variety of pathogens including *Yersinia pestis* (the causative agent of bubonic plague), *Rickettsia* species (e.g. flea-borne spotted fever and murine typhus) and *Bartonella* species (bartonellosis, including cat scratch disease), all of which are considered emerging or re-emerging diseases of humans worldwide (Azad et al*.,* 1997; Bitam et al*.,* 2010).

The co-evolution of Australian marsupials and their fleas has been discussed and elucidated in the context of zoogeography (Dunnet and Mardon. 1974; Lewis et al*.,* 1993; Whiting et al*.,* 2008) and the taxonomic classification of fleas associated with Australian marsupials has provided evidence for the origin and distribution of metatherian hosts prior to the separation of Gondwana (Dunnet and Mardon. 1974; Whiting et al*.,* 2008). Morphological identification of fleas is still the major tool for flea taxonomy, which is used for the study of phylogenetic relationships. However, this approach is limited by the requirement for skilled entomologists to elucidate flea morphology (Whiting et al*.,* 2008; Bitam et al*.,* 2010).

A recent study used multilocus sequence analysis of four genes (18S rRNA, 28S rRNA, cytochrome oxidase II and elongation 1-alpha) to examine phylogenetic relationships in the order Siphonaptera (Whiting et al*.,* 2008). Since then a re-evaluation of flea taxonomy using molecular tools has begun to explore the relationship between fleas as vectors of

infectious agents and their mammalian hosts (Bitam et al., 2010). Distribution of arthropod-borne pathogens is influenced by the host range of arthropod vectors (Chomel et al., 2009). The presence of co-evolution between marsupial hosts and their fleas in the same habitats has been reviewed (Dunnet and Mardon. 1974; Lewis et al., 1993; Whiting et al., 2008), but to the authors' knowledge co-evolution of hosts, vectors and *Bartonella* species has not been demonstrated. Recently, new *Bartonella* species and new candidate *Bartonella* species have been detected in marsupials, mammals and their fleas in Australia (Fournier et al., 2007; Gundi et al., 2009; Kaewmongkol et al., 2011a; 2011b (in press)). Three *Bartonella* species, *B. queenslandensis*, *B. rattaustraliani* and *B. coopersplainsensis,* were previously isolated from native placental mammals (rodents) in Queensland, eastern Australia (Gundi et al., 2009). *Bartonella australis* was isolated from marsupials (eastern grey kangaroos) in eastern Australia (Fournier et al., 2007). In addition, three new candidate *Bartonella* species, *Candidatus* B. antechini, *Candidatus* B. woyliei and *Candidatus* B. bandicootii were detected in fleas and ticks collected from native marsupials in Western Australia (Kaewmongkol et al., 2011a; 2011b (in press)). Two zoonotic *Bartonella* species, *B. henselae* and *B. clarridgeiae* were also detected in fleas collected from red foxes in Western Australia (Kaewmongkol et al., 2011c (in press)). It has been suggested that the close relationship between *Bartonella* species and fleas may be a contributing factor to the host specificity of these *Bartonella* species in Australian marsupials (Kaewmongkol et al., 2011a; 2011b (in press)). We examine this concept further in the present study by evaluating the genetic relationships among flea species harbouring a variety of *Bartonella* species and their marsupial hosts in Australia.

2. Materials and methods

2.1 Flea samples and morphological identification

Ten species of fleas were collected from a variety of locations in Western Australia (Table 1). Species of fleas were characterized by light microscopy using the standard key for Australian fleas (Dunnet and Mardon. 1974).

2.2 DNA extraction, PCR and phylogenetic analysis

DNA was extracted from fleas using a DNeasy Blood and Tissue Kit (Qiagen, Maryland, USA) according to the manufacturer's protocol. DNA samples extracted from these fleas were screened for *Bartonella* species using PCR as described in previous studies (Kaewmongkol et al*.,* 2011a; 2011b (in press); 2011c (in press)).

For amplification of flea DNA, PCR primers for the 18S rRNA gene were designed based on *Ctenocephalides canis* DNA sequences (GenBank accession no. AF423914) and consisted of 18S-F (5' GATCGTACCCACATTACTTG 3') and 18S-R (5' AAAGAGCTCTCAATCTGTCA 3'). PCR reactions for the 18S rRNA gene were performed using 1 µL of DNA in a 25 µL reaction containing 1 x PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM of each primer and 0.02 U/µL TAQ-Ti (hot start) Taq DNA polymerase (Fisher Biotech Australia, Wembley, W.A., Australia). The cycling conditions consisted of a pre-PCR step of 96ºC for 2 minutes, followed by 45 cycles of 94ºC for 50 seconds, 55ºC for 60 seconds and an extension of 72ºC for 90 seconds, with a final extension of 72ºC for 10 minutes. PCR primers for the cytochrome oxidase subunit I (the *COI* gene), LCO1490: 5 'GGTCAACAAATCATAAAGATATTGG 3' and HC02198: 5' TAAACTTCAGGGTGACCAAAAAATCA 3' were used as per a previous study (Folmer et al*.,* 1994). The PCR reaction for the *COI* gene were performed using 1 µL of DNA in a 25 µL reaction containing 1 x PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM of each primer and 0.02 U/µL TAQ-Ti (hot start) Taq DNA polymerase (Fisher Biotech Australia, Wembley, W.A., Australia). The cycling conditions consisted of a pre-PCR step of 96ºC for 2 minutes, followed by 40 cycles of 94ºC for 30 seconds, 50ºC for 30 seconds and an extension of 72ºC

for 60 seconds with a final extension of 72ºC for 7 minutes. PCR products from all genes were purified from agarose gel slices using an UltraClean™ 15 DNA Purification Kit (MO BIO Laboratories Inc. West Carlsbad, California, USA). Sequencing was performed using an ABI Prism™ Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California, USA) on an Applied Biosystems 3730 DNA Analyzer, following the manufacturer's instructions. Nucleotide sequences generated for 2 loci were analysed using Chromas lite version 2.0 (http://www.technelysium.com.au) and aligned with reference sequences from GenBank using Clustal W (http://www.clustalw.genome.jp). Phylogenetic analysis was conducted on concatenated 18S rRNA and *COI* sequences. Distance and maximum-parsimony were conducted using MEGA version 4.1 (MEGA4.1: Molecular Evolutionary Genetics Analysis software, Arizona State University, Tempe, Arizona, USA), based on evolutionary distances calculated with the Kimura's distance and grouped using Neighbour-Joining. Bootstrap analyses were conducted using 10,000 replicates to assess the reliability of inferred tree topologies. DNA sequences generated in the present study were submitted to GenBank (see Table 2 for accession numbers).

3. Results

The phylogenetic relationships among the 10 species of fleas, based on concatenated sequences from 2 loci are shown in Fig. 1. Genetic clustering of these fleas correlated with the Family classification based on morphological identification (Dunnet and Mardon, 1974). The 10 species of fleas were categorized into 3 Families; Pulicidae (*Ctenocephalides felis*, *Spilopsyllus cuniculi*, *Echidnophaga myrmecobii*, *Echidnophaga gallinacea*, and an unidentified *Echidnophaga* sp.), the Stephanocircidae (*Stephanocircus pectinipes* and *Stephanocircus dasyuri*) and the Pygiopsyllidae (*Pygiopsylla hilli*, *Pygiopsylla tunneyi* and *Acanthopsylla jordani*).

The corresponding mammalian hosts from which these fleas were collected were included in the concatenated tree (Fig. 1). These results demonstrate the relationship between fleas in the Family Pulicidae and a wide host range of introduced (i.e. non-native) mammalian species; red foxes, feral cats and rabbits (Fig. 1). In contrast, association between mammalian hosts and fleas in the Families Stephanocircidae and Pygiopsyllidae, are restricted to Australian rodents (bush rats) and marsupials respectively (Fig. 1).

The *Bartonella* species detected in these fleas were also included in the concatenated tree (Fig. 1) and indicates that two zoonotic *Bartonella* species (*B. henselae* and *B. clarridgeiae*) are distributed among flea species belonging to Family Pulicidae, which are known to share a wide variety of mammalian hosts. However, restriction of host range was evident in *Bartonella* species detected in marsupials as both the fleas and the *Bartonella* species were genetically distinct (Fig. 1 and 2) from those infecting non-Gondwana origin hosts. This study also demonstrates the relationship between *Bartonella* species (*B. rattaustraliani* and *B. coopersplainsensis*) and fleas in Family Stephanocircidae from a native Australian rodent, *Rattus fuscipes* (Fig. 1). The genetic position of *B. rattaustraliani* and *B. coopersplainsensis* is also separated from other *Bartonella* species from rodents in other geographical areas (Fig. 2).

4. Discussion

Co-evolution between *Bartonella* species and flea vectors has been proposed previously for flea species infesting rodents and felids (Chomel et al*.,* 1996; 2009; Bown et al*.,* 2004) and the demonstration of host specificity between *B. washoensis* and squirrels has also been reported (Inoue et al*.,* 2010). Moreover, a geographical relationship was noted for *B. grahamii* and various rodent hosts in Japan, China (represent Asian group), Canada, UK, Russia and USA (represent American/European group) (Inoue et al*.,* 2009; Berglund et al*.,* 2010). In the present study, the close association between Australian marsupials, their fleas

and *Bartonella* species suggests adaptation by *Bartonella* species to a specific ecological niche which is comprised of specific placental or marsupial hosts and specific flea vectors. The *Bartonella* species in the marsupial cluster, reported in previous studies (Kaewmongkol et al., 2011a; 2011b (in press)), appear to have evolved separately in marsupials and their fleas within Australia.

The introduction of mammalian pest species has been mooted to interfere with native host-bacteria interactions (Telfer et al., 2005; 2007; 2010; Chomel et al., 2009) and the impact of the red fox on Australia's native animals has been discussed in the context of the spread of diseases and parasites (Glen et al., 2005). Our finding of *B. henselae* and *B. clarridgeiae* in flea species of the Family Pulicidae only may reflect the limited number of samples examined, but is consistent with these bacteria being co-introduced with pest species in post-colonial Australia. Alternatively, these two zoonotic *Bartonella* species could be distributed through a wide variety of native species via a broad range of the Pulicidae fleas. Therefore, genetic segregation of *Bartonella* species in marsupials could provide an opportunity to study *Bartonella* genetics in the context of host and vector specificity.

Three *Bartonella* species; *B. queenslandensis*, *B. rattaustraliani* and *B. coopersplainsensis* were isolated from native placental mammals (rodents) including *Rattus fuscipes*, *Rattus leucopus*, *Rattus sordidus* (*Rattus conatus*), *Rattus tunneyi*, *Uromys caudimaculatus* and unidentified *Melomys* spp. in Queensland, eastern Australia (Gundi et al., 2009). The evolution lineage of the genera *Uromys* and *Melomys* in Australia is still largely unknown. However, the evolutionary relationships within the genus *Rattus* has been defined (Robins et al., 2007; 2008; 2010). All of these *Rattus* species were grouped into the Australo-Papuan clade which is one of two major groups of the genus *Rattus*. The other major group is the Asian clade which includes *Rattus rattus*, *Rattus norvegicus* and *Rattus exulans* (Robins et al., 2007; 2008; 2010). The divergence of the genus *Rattus* into these two

clades occurred approximately 2.7 million years ago (Robins et al., 2010). *Rattus fuscipes*, a rodent host for Stephanocircidae fleas in this study, is the oldest lineage of the Australo-Papuan clade in Australia The concatenated phylogenetic analysis of multigene analysis revealed that *B. queenslandensis* was closely related to *Bartonella* species from other rodent isolates (Gundi et al., 2009) (Fig 2). In contrast to *B. queenslandensis, B. rattaustraliani* and *B. coopersplainsensis* were less related to other *Bartonella* species isolated from rodents (Gundi et al., 2009) (Fig. 2). In the present study, genetic clustering of fleas in the Family Stephanocircidae correlated with the morphology classifications from previous studies (Traub et al., 1972; Dunnet and Mardon. 1974; Lewis et al., 1993) and also correlates well with genetic classification of fleas in the study by Whiting et al., (2008). Fleas in the Family Stephanocircidae are well discriminated from other flea families using both morphological and genetic classifications (Traub et al., 1972; Dunnet and Mardon. 1974; Lewis et al., 1993; Whiting et al., 2008). In addition, these fleas are unique and most likely originated in Australia prior to the separation of the continents (Whiting et al., 2008). In the present study, the compatibility between *B. rattaustraliani* and *B. coopersplainsensis* and Australian fleas from the Family Stephanocircidae was evident (Fig. 1) and this relationship could explain the genetic positioning of *B. rattaustraliani* and *B. coopersplainsensis*, which are less related to other *Bartonella* species isolated from placental mammals (rodents) outside Australia. It can be hypothesized that fleas in the Family Stephanocircidae may play an important role in Australia in the evolution of *Bartonella* species in Australian rodents. Therefore, *B. queenslandensis* may have evolved with non-native rodents, which were introduced by European colonists in the 19th century.

The study of Australian flea morphology and phylogeny has provided evidence for the origin and distribution of marsupial ancestors (Dunnet and Mardon. 1974; Whiting et al., 2008). Investigation of the fossil record reveals that ancestral marsupials dispersed from

Australia (east Gondwana) to South America (west Gondwana) (Beck et al., 2008). Dispersal directions of marsupial ancestors have also been associated with the dispersal of fleas in the Family Pygiopsyllidae into New Guinea and Family Stephanocircidae into South America (Dunnet and Mardon. 1974; Whiting et al., 2008). The investigation of *Bartonella* species in these two Families of fleas in other geographical areas would be useful for the study of the origin of metatherians and their radiation across the continents. Furthermore, deeper study of the *Bartonella* genome may also provide insights into the evolution of these enigmatic bacteria and their mammal and vector hosts.

Acknowledgements

We would like to thank Louise Pallant, Yazid Abdad, Michael Banazis, Rongchang Yang, and Josephine Ng for their technical support.

References

Azad, A. F., Radulovic, S., Higgins, J. A., Noden, B. H., and Troyer, J. M. 1997. Flea-borne rickettsioses: ecologic considerations. Emerg. Infect. Dis. 3(3), 319-27.

Beck, R. M., Godthelp, H., Weisbecker, V., Archer, M., and Hand, S. J. 2008. Australia's oldest marsupial fossils and their biogeographical implications. PLoS One 3(3), e1858.

Berglund, E. C., Ellegaard, K., Granberg, F., Xie, Z., Maruyama, S., Kosoy, M. Y., Birtles, R. J., and Andersson, S. G. 2010. Rapid diversification by recombination in *Bartonella grahamii* from wild rodents in Asia contrasts with low levels of genomic divergence in Northern Europe and America. Mol. Ecol. 19(11), 2241-55.

Bitam, I., Dittmar, K., Parola, P., Whiting, M. F., and Raoult, D. 2010. Fleas and flea-borne diseases. Int. J. Infect. Dis. 14(8), e667-76.

Bown, K. J., Bennet, M., and Begon, M. 2004. Flea-borne *Bartonella grahamii* and

Bartonella taylorii in bank voles. Emerg. Infect. Dis. 10(4), 684-7.

Chomel, B. B., Boulouis, H. J., Breitschwerdt, E. B., Kasten, R. W., Vayssier-Taussat, M., Birtles, R. J., Koehler, J. E., and Dehio, C. 2009. Ecological fitness and strategies of adaptation of *Bartonella* species to their hosts and vectors. Vet. Res. 40(2), 29.

Chomel, B. B., Kasten, R. W., Floyd-Hawkins, K., Chi, B., Yamamoto, K., Roberts-Wilson, J., Gurfield, A. N., Abbott, R. C., Pedersen, N. C., and Koehler, J. E. 1996. Experimental transmission of *Bartonella henselae* by the cat flea. J. Clin. Microbiol. 34(8), 1952-6.

Dunnet, G. M., Mardon, D. K. 1974. A Monograph of Australian Fleas (Siphonaptera). Australian Journal of Zoology Supplementary Series 22, 1-273.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3(5), 294-9.

Fournier, P. E., Taylor, C., Rolain, J. M., Barrassi, L., Smith, G., and Raoult, D. 2007. *Bartonella australis* sp. nov. from kangaroos, Australia. Emerg. Infect. Dis. 13(12), 1961-2.

Glen, A. S., and Dickman, C. R. 2005. Complex interactions among mammalian carnivores in Australia, and their implications for wildlife management. Biol. Rev. Camb. Philos. Soc. 80(3), 387-401.

Gundi, V. A., Taylor, C., Raoult, D., and La Scola, B. 2009. *Bartonella rattaustraliani* sp. nov., *Bartonella queenslandensis* sp. nov. and *Bartonella coopersplainsensis* sp. nov., identified in Australian rats. Int. J. Syst. Evol. Microbiol. 59(Pt 12), 2956-61.

Inoue, K., Kabeya, H., Hagiya, K., Kosoy, M. Y., Une, Y., Yoshikawa, Y., and Maruyama, S. 2010. Multi-locus sequence analysis reveals host specific association between *Bartonella washoensis* and squirrels. Vet. Microbiol. 148(1), 60-5.

Inoue, K., Kabeya, H., Kosoy, M. Y., Bai, Y., Smirnov, G., McColl, D., Artsob, H., and Maruyama, S. 2009. Evolutional and geographical relationships of *Bartonella grahamii* isolates from wild rodents by multi-locus sequencing analysis. Microb. Ecol. 57(3)**,** 534-41.

Kaewmongkol, G., Kaewmongkol, S., Owen, H., Fleming, P. A., Adams, P. J., Ryan, U., Irwin, P. J., and Fenwick, S. G. 2011a. *Candidatus* Bartonella antechini: a novel *Bartonella* species detected in fleas and ticks from the yellow-footed antechinus (*Antechinus flavipes*), an Australian marsupial. Vet. Microbiol. 149(3-4)**,** 517-21.

Kaewmongkol, G., Kaewmongkol, S., Owen, H., Fleming, P. A., Adams, P. J., Ryan, U., Irwin, P. J., Fenwick, S. G., Burmej, H., Bennett, M. D., and Wayne, A. F. 2011b. Diversity of *Bartonella* species detected in arthropod vectors from animals in Australia. Comparative Immunology, Microbiology & Infectious Diseases. in press.

Kaewmongkol, G., Kaewmongkol, S., Fleming, P. A., Adams, P. J., Ryan, U., Irwin, P. J., and Fenwick, S. G. 2011c. Zoonotic *Bartonella* species in Fleas and Blood from Red Foxes in Australia. Vector-Borne and Zoonotic Diseases. in press.

Lewis, R. E. 1993. Notes on the geographical distribution and host preferences in the order Siphonaptera. Part 8. New taxa described between 1984 and 1990, with a current classification of the order. J. Med. Entomol. 30(1)**,** 239-56.

Robins, J. H., Hingston, M.,Matisoo-Smith, E., Ross, H. A. 2007. Identifying *Rattus* species using mitochondrial DNA. Mol. Ecol. Notes **7,** 717-729

Robins, J. H., McLenachan, P. A., Phillips, M. J., Craig, L., Ross, H. A., and Matisoo-Smith, E. 2008. Dating of divergences within the *Rattus* genus phylogeny using whole mitochondrial genomes. Mol. Phylogenet. Evol. 49(2)**,** 460-6.

Robins, J. H., McLenachan, P. A., Phillips, M. J., McComish, B. J., Matisoo-Smith, E., and Ross, H. A. 2010. Evolutionary relationships and divergence times among the native

rats of Australia. BMC Evol. Biol. 10, 375.

Telfer, S., Begon, M., Bennett, M., Bown, K. J., Burthe, S., Lambin, X., Telford, G., and Birtles, R. 2007. Contrasting dynamics of *Bartonella* spp. in cyclic field vole populations: the impact of vector and host dynamics. Parasitology 134(Pt 3)**,** 413-25.

Telfer, S., Bown, K. J., Sekules, R., Begon, M., Hayden, T., and Birtles, R. 2005. Disruption of a host-parasite system following the introduction of an exotic host species. Parasitology 130(Pt 6)**,** 661-8.

Telfer, S., Lambin, X., Birtles, R., Beldomenico, P., Burthe, S., Paterson, S., and Begon, M. 2010. Species interactions in a parasite community drive infection risk in a wildlife population. Science 330(6001)**,** 243-6.

Traub, R. 1972. Notes on zoogeography, convergent evolution and taxonomy of fleas (Siphonaptera), based on collections from Gunong Benom and elsewhere in South-East Asia. III. Zoogeography. Bull. Br. Mus. (Nat. Hist.) Zool., Lond. 23, 291-450.

Whiting, M. F., Whiting, A. S., Hastriter, M. W., Dittmar, de la Cruz K. 2008. A molecular phylogeny of fleas (Insecta: Siphonaptera): origins and host associations. Cladistics 24**,** 1-30.

Table 1

Flea species collected from a variety of locations in Western Australia

Table 2

GenBank accession numbers of flea species from animals in Australia

Fig. 1. Neighbor-Joining concatenated phylogenetic tree of the 18S rRNA, and *COI* genes (1,820-bp nucleotide) of flea species and their associated *Bartonella* species from marsupials

and other mammals in Australia. Percentage bootstrap support (>40%) from 10,000 pseudoreplicates is indicated at the left of the supported node. The tree is rooted using *Calliphora vomitoria* (fly) as an outgroup.

Fig. 2. Neighbor-Joining concatenated phylogenetic tree of 16S rRNA, *gltA*, *ftsZ*, *rpoB*, and the ITS region of *Bartonella* species showing the separate clustering of *Bartonella* spp. from Australian marsupials and *B. rattaustraliani* and *B. coopersplainsensis* from Australian rodents. Percentage bootstrap support (>60%) from 1,000 pseudoreplicates is indicated at the left of the supported node (Kaewmongkol et al., 2011b (in press)).

Table 1

Flea species collected from a variety of locations in Western Australia

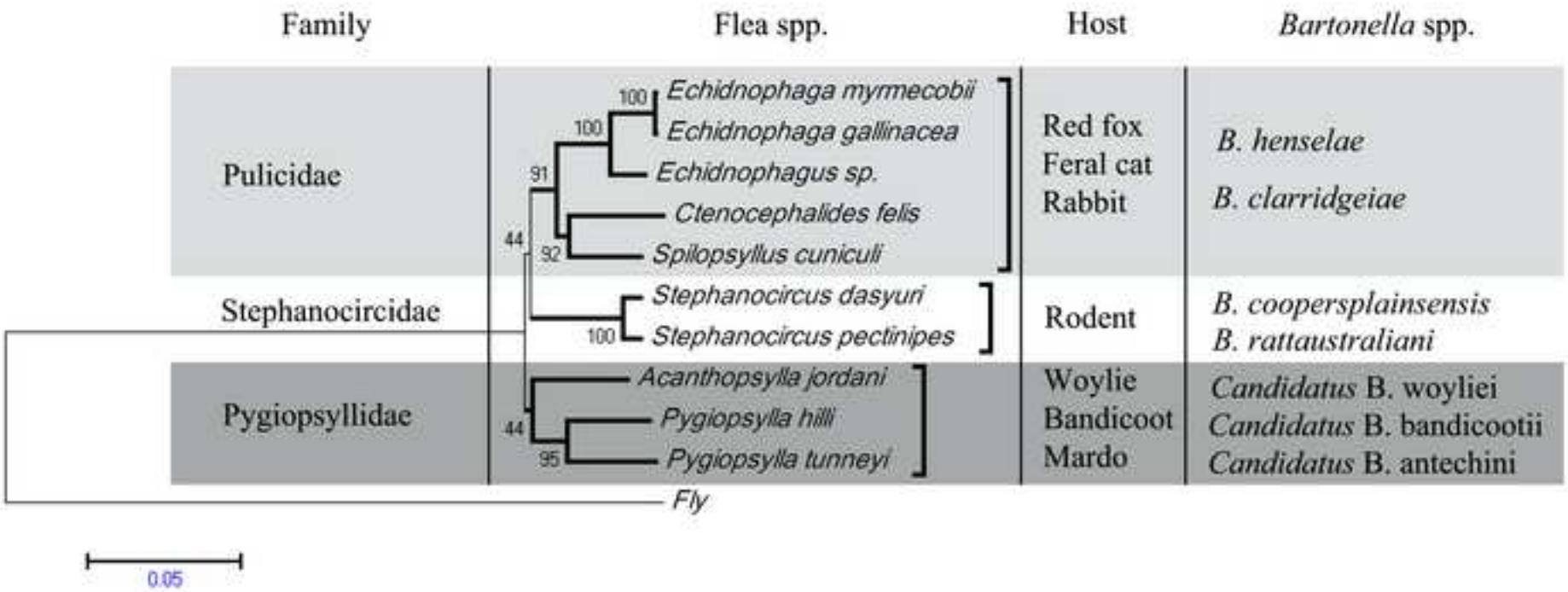
Species	Host		Location		Year	Reference
Pygiopsylla hilli	Woylie [m] (*Bettongia penicillata*)	Southwest Forest	Keninup Balban Karakamia Sanctuary	34°2′S, 116°37′E 34°5′S, 116°35′E 31°48′S, 116°14′E	2003-2004	Kaewmongkol et al., 2011b (in press)
Stephanocircus pectinipes *Stephanocircus dasyuri*	Rodent [M] (*Rattus fuscipes*)	South Coast	Fitzgerald River National Park	33°56′S, 119°37′E		
Pygiopsylla tunneyi	Western barred bandicoot (*Perameles bougainville*) [m]		Bernier and Dorre Islands	25°7′S, 113°6′E 24°51′S, 113°8′E	2005-2007	Kaewmongkol et al., 2011b (in press)
Acanthopsylla jordani	Mardo (*Antechinus flavipes*) [m]	Southwest	The areas surrounding the town of Dwellingup	32°38′S, 116°5′E	2003-2004	Kaewmongkol et al., 2011a
Ctenocephalides felis *Spilopsyllus cuniculi* *Echidnophaga myrmecobii* *Echidnophaga gallinacea* *Echidnophaga* sp.	Red fox [M] (*Vulpes vulpes*) Feral cat [M] (*Felis catus*) Rabbit [M] (*Oryctolagus cuniculus*)	Southwest	The areas surrounding the towns of Katanning and Boyup Brook	33°41′S, 117°34′E 33°50′S, 116°23′E	2010	Kaewmongkol et al., 2011c (in press)

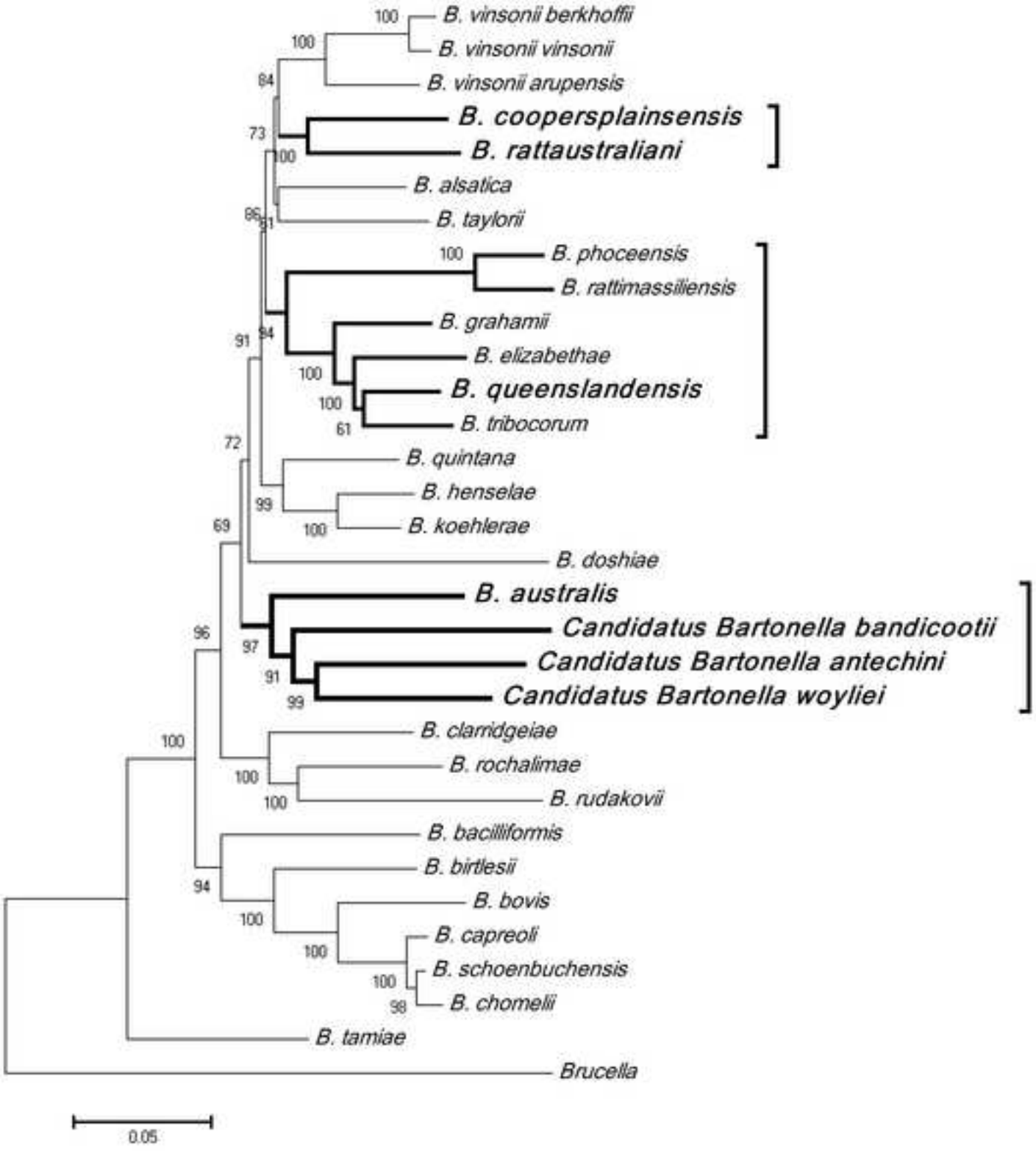
[m] Marsupial mammals.
[M] Placental mammals.

Table 2

GenBank accession numbers of flea species from animals in Australia

Family	Species	Host	*Bartonella* species	GenBank accession numbers	
				18S rRNA	Cytochrome oxidase I
Pygiopsyllidae	*Pygiopsylla hilli*	Woylie (*Bettongia penicillata*)	*Candidatus* B. woyliei	JN008926	JN008915
	Pygiopsylla tunneyi	Western barred bandicoot (*Perameles bougainville*)	*Candidatus* B. bandicootii	JN008933	JN008924
	Acanthopsylla jordani	Mardo (*Antechinus flavipes*)	*Candidatus* B. antechini	JN008925	JN008916
Stephanocircidae	*Stephanocircus pectinipes*	Rodent (*Rattus fuscipes*)	*B. coopersplainsensis* *B. rattaustraliani*	JN008932	JN008923
	Stephanocircus dasyuri			JN008930	JN008920
Pulicidae	*Ctenocephalides felis*	Red fox (*Vulpes vulpes*)	*B. henselae* *B. clarridgeiae*	JN008927	JN008917
	Spilopsyllus cuniculi	Feral cat (*Felis catus*)		JN008928	JN008918
	Echidnophaga myrmecobii	Rabbit (*Oryctolagus cuniculus*)		JN008929	JN008919
	Echidnophaga gallinacea			JN008931	JN008921
	Echidnophaga sp.			JN008934	JN008922





Highlights

Association of *Bartonella* species, Australian fleas and Australian faunas