

Losing and Gaining Endosymbionts: Evolutionary History of Blood Feeding Parasites and their Bat Hosts



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Introduction

Microbial associates are ubiquitous in ectoparasitic bat flies (Figure 1, C) of the families Nycteribiidae and Streblidae (Diptera). Among other clearly mutualist microbes (e.g., *Arsenophonus* spp.)¹, we regularly detect *Bartonella* spp., whose function in flies is unknown. However, a growing number of bartonellae (alpha-proteobacteria) have been identified as causative agents for a broadening spectrum of mammalian diseases (incl. humans), emphasizing their medical importance². For instance, *Bartonella quintana* causes Trench Fever. Recently, research has confirmed the presence of these pathogens in bats, yet studies of their potential vectors – the bat flies – are scarce³. Because bat flies are blood feeders, they may be able to transfer pathogens (microbes) between hosts.

Questions

Bats (Chiroptera) constitute 25% of mammals, and a large diversity of bats and bat flies occurs throughout the Philippine Archipelago. Because of prior anecdotal records of bartonellae in bat flies, we hypothesized that Philippine genera may harbor bartonellae on a larger scale than previously thought. Specific questions were:

Q1) How prevalent are bartonellae in Philippine bat fly genera, and does this provide information about gains and losses of bartonellae throughout fly evolution?

Q2) What is the distribution of bartonellae throughout fly developmental stages (e.g., pupa, adult), and how does that relate to microbe-fly relations?

Q3) What is the general history of bartonellae in Philippine bat flies, and are there any host and/or biogeographic patterns?

Materials and Methods

Fly specimens (500+, adults and pupae) were collected from all known Philippine genera (10; 2 families) from 3 provinces on Luzon Island, Philippines, stored in 99% ethanol, and identified using available taxonomic keys. Bat fly total genomic DNA was extracted from whole fly specimens and PCR reactions were run on microbial *gltA* genes using established primers and protocols⁴. PCR products were cloned (if necessary) and sequenced. During sequence analysis Philippine samples were aligned with *gltA* gene results from bat and bat fly samples from 15 different countries as well as representative specimens from other published sequences in GenBank (NCBI Database). Trees were rooted to *Arsenophonus* spp. *gltA*, another distantly related microbe. Phylogenetic analyses were conducted using Maximum Likelihood (ML) as implemented in PhyML⁵, with evolutionary models (jModelTest)⁶. Nodal support was assessed using bootstrapping methods (1000 iterations).

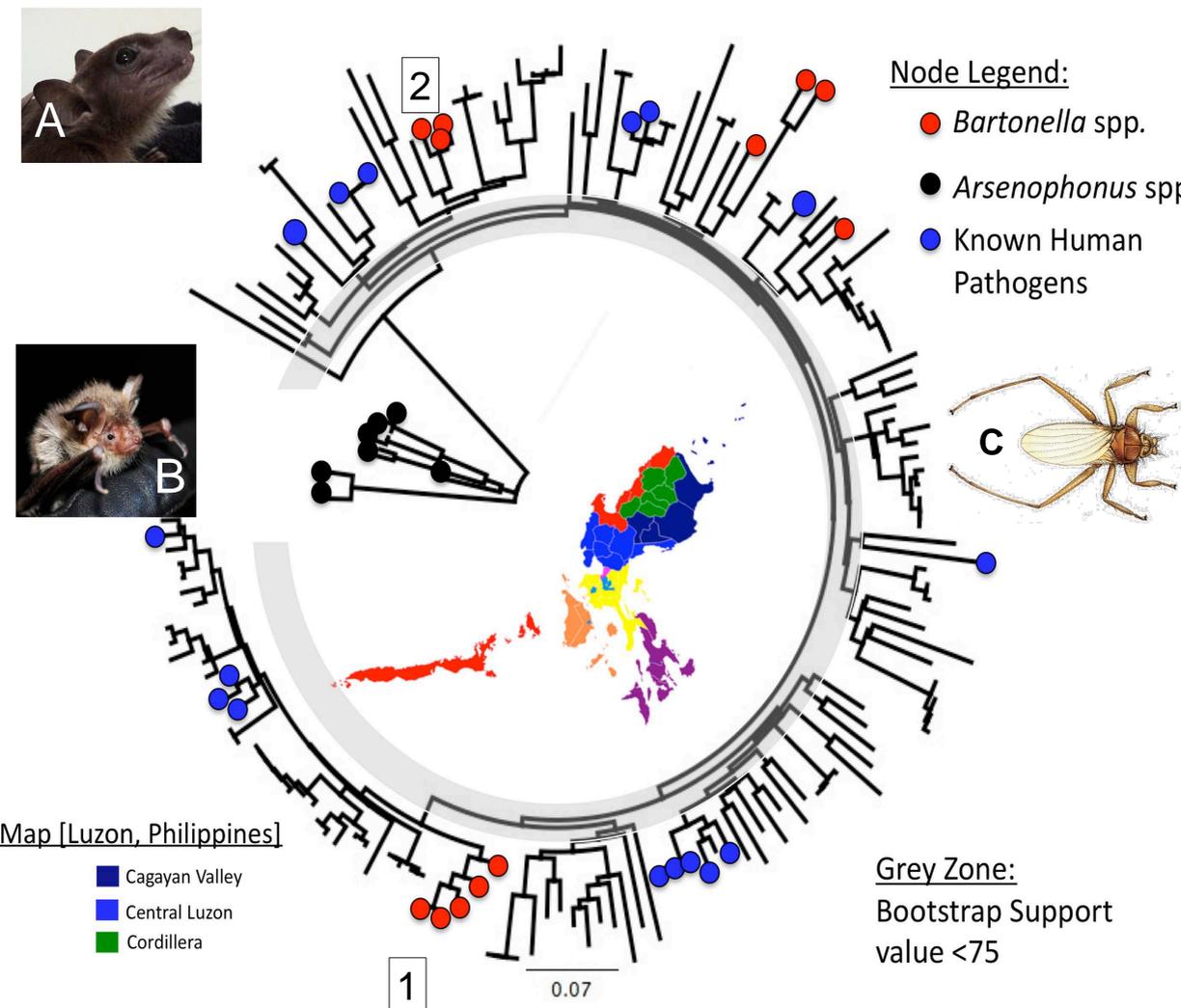


Figure 1. Rooted (black dots) ML Topology of *Bartonella* spp. *gltA* from 179 genotypes, from bat flies. Red dots indicate *Bartonella* spp. *gltA* genes generated from Philippine samples in this effort. Numbers indicate location [Group 1: Cordillera; Group 2: Central Luzon]. A. Mega-bat. B. Micro-bat (Chiroptera = hosts of bat flies). C. bat fly.

Results

New bartonellae genotypes were detected in 50% of all specimens in this effort. Both adults and pupae as well as 40% of all genera were positive for bartonellae. The positive flies came from 5 genera of Mega- (A), and Micro-bats (B). jModelTest identified the GTR+I+G model as the best fit for *gltA*. In line with previous analyses, the genus *Bartonella* sp. was recovered as a monophyletic clade (Figure 1). Based on the ML topology, Philippine samples (Red) nest in various locations among the other *Bartonella* sp. *gltA* genotypes. While the *gltA* gene has good discriminatory power to delimit genotypes and species (tips of the tree), the basal relationships (grey zone), are poorly supported (Figure 1). This mirrors previous analyses, and is in part due to saturation in the third codon position. Interestingly, many Philippine bat fly bartonellae intersperse with clades of known human pathogens (Blue). Some sub-clades seemingly correlate to sampling location, irrespective of the evolutionary relationships of their host flies (1: Cordillera; 2: Central Luzon; Figure 1). Others do not follow this pattern (red dots with no number), and seem to cluster by generic relation.

Discussion and Future Studies

This study significantly increases the number of known bartonellae genotypes from the Philippines in general, and Luzon in particular (Q1). It also considerably extends the known global and specific geographic and host ranges (Q3). The diversity of detected genotypes suggests that bat flies may serve as reservoirs for different types of bartonellae² (Q1). The wide distribution of bartonellae in bat flies throughout the tree as well as the basal position of some of our samples (Group 2, Figure 1) suggests an evolutionarily early association (gain) of bartonellae with bat flies (Q1). Not all tested genera had bartonellae which could be due either to secondary losses of this association through time (Q1) or to never associating with some taxa in the first place. The detection of *Bartonella* interspersed with related bat fly species is indicative of co-evolutionary scenarios (red dots with no number, Figure 1). The grouping of genotypes by location (Groups 1, 2; Figure 1) also suggests an underlying biogeographic pattern⁷. The detection of bartonellae across fly developmental stages indicates vertical transmission. Vertical transmission is more common in mutualist (beneficial) symbionts. However, it also supports the notion of vector competency (bartonellae survive, replicate and are transmitted by the flies they infect), which is more in line with a pathogenic function⁸ (Q2). The role of *Bartonella* sp. in flies is difficult to determine from the current data, but preliminary studies suggest a facultative association because bartonellae do not appear in all species (see Results). Alternatively, because of a previously observed skewed sex ratio in flies⁹, and the close relationship of bartonellae to *Wolbachia* - a reproductive manipulator - bartonellae may be pathogenic (parasitic) in these flies (Q2). Given the genetic variation of genotypes in our samples, a large, yet to be discovered diversity is likely. Further studies are needed to fully understand the function of this microbe across this vertebrate (bats)-invertebrate (bat fly) system.

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