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Wolbachia Infections in the Cimicidae: Museum Specimens as an Untapped Resource for Endosymbiont Surveys

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***Wolbachia* spp. are obligate maternally inherited endosymbiotic bacteria that infect diverse arthropods and filarial nematodes. Previous microscopic and molecular studies have identified *Wolbachia* in several bed bug species (Cimicidae), but little is known about how widespread *Wolbachia* infections are among the Cimicidae. Because cimicids of non-medical importance are not commonly collected, we hypothesized that preserved museum specimens could be assayed for *Wolbachia* infections. For the screening of museum specimens, we designed a set of primers that specifically amplify small diagnostic fragments (130 to 240 bp) of the *Wolbachia* 16S rRNA gene. Using these and other previously published primers, we screened 39 cimicid species (spanning 16 genera and all 6 recognized subfamilies) and 2 species of the sister family Polyctenidae for *Wolbachia* infections using museum and wild-caught material. Amplified fragments were sequenced to confirm that our primers were amplifying *Wolbachia* DNA. We identified 10 infections, 8 of which were previously undescribed. Infections in the F supergroup were common in the subfamily Cimicinae, while infections in the A supergroup were identified in the subfamilies Afrocimicinae and Haematosiphoninae. Even though specimens were degraded, we detected infections in over 23% of cimicid species. Our results indicate that *Wolbachia* infections may be common among cimicids and that archived museum material is a useful untapped resource for invertebrate endosymbiont surveys. The new screening primers listed in this report will be useful for other researchers conducting *Wolbachia* surveys with specimens with less-than-optimum DNA quality.**

Wolbachia spp. are endosymbiotic bacteria that have been described with a diverse range of arthropods and filarial nematodes (8, 11, 18, 19, 21, 25, 27, 28). Eight major *Wolbachia* "supergroups" (A to H) exist based on phylogenetic clustering of *FtsZ* gene sequences (11). A, B, and E infect diverse arthropods; C and D infect nematodes; G infects spiders; H infects termites; and F infects both arthropods and nematodes (4, 5, 8, 11, 18, 19, 21, 25, 27, 28). *Wolbachia* infections are commonly associated with diverse host reproductive alterations, including cytoplasmic incompatibility, feminization, male killing, parthenogenesis, increased or decreased fitness, and obligate symbiosis (21). Because of the phenotypes induced by these infections, it has been suggested that the manipulation of endosymbiotic bacteria can be used as a novel method for the biocontrol of pest arthropods of medical, veterinary, and agricultural importance (3, 16, 17, 20, 22, 29, 30).

The Cimicidae (bed bugs) are obligatory hematophagous ectoparasites of birds, bats, and humans (24). *Wolbachia*-like bacterial inclusions were observed several decades ago in the gonads, spermalege (i.e., organ of Berlese), gut, Malpighian tubules, and hemolymph of the cimicids *Cimex lectularius* and *Oeciacus hirundinis* (1, 24). Additionally, similar organisms have been described from the bacteriomes (i.e, mycetomes) of *C. lectularius* (5, 26). More recently, modern molecular methods were used to conclusively identify *Wolbachia* symbionts in *C. lectularius* and *Oeciacus vicarius* (8, 18), which were determined to be closely related to one another in the F supergroup (11, 18). However, except for these two species, nothing is known about the distribution of *Wolbachia* infections among the family Cimicidae.

We undertook a PCR-based survey to screen for *Wolbachia* infections in the Cimicidae. Studies of this nature are complicated by the fact that, apart from species of medical importance, cimicids that feed on nonhuman hosts are not frequently collected. Non-medically important cimicids are obtained primarily incidentally during vertebrate ectoparasite surveys and are often preserved in ethanol and archived in museum collections. Museum specimens have previously been used for molecular surveys of bacteria, such as *Borrelia*, *Helicobacter*, and *Mycobacterium* spp. (2, 7, 9, 12, 13, 15). We therefore hypothesized that ethanol-preserved museum material could be used in a similar manner for *Wolbachia* surveys. Despite degraded DNA in many specimens, it was possible to amplify and sequence diagnostic fragments of the *Wolbachia* 16S rRNA gene in both wild-caught and preserved cimicid specimens. In a screen of 39 species of Cimicidae and 2 species in the sister family Polyctenidae (24), 10 *Wolbachia* infections were identified, 8 of which were previously undescribed. *Wolbachia* infections were detected in ethanol-preserved museum specimens up to 48 years old. Our results suggest that *Wolbachia* infections may be common in the family Cimicidae and that

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TABLE 1. Collection information and sample sizes for specimens assayed in this study

Species	No. of specimens	Source	Collection date	Collection locality	Reference[b]
Afrocimex constrictus	5[e]	Wild[f]	March 2005	Mt. Elgon, Kenya	
Aphrania vishnou	2[e]	Museum[g]	1952[a]	Phnom Penh, Cambodia[a]	24
Bucicimex chilensis	1[e]	Museum[g]	January 1962	Lab colony of unknown origin	
Cacodmus vicinus	1[e]	Museum[g]	April 1959	Giza, Egypt	
Cimex adjunctus	2[e]	Museum[f]	July 2001	Hillsboro County, N.H.	
Cimex antennatus	6[e]	Museum[g]	July 1963	Siskiyou County, Calif.	
Cimex brevis	6[e]	Museum[g]	Before 1966[a]	Staples, Minn.	24
Cimex columbarius	2[e]	Museum[g]	July 1958[a]	Island of Korpo, Finland[a]	23, 24
Cimex hemipterus	2[e]	Museum[g]	Before 1966[a]	Taiwan	24
Cimex incrassatus	2[e]	Museum[f]	July 1997	Orange County, Calif.	
Cimex insuetus	8[e]	Museum[g]	Before 1966[a]	Saraburi, Thailand	24
Cimex latipennis	8[e]	Museum[g]	Before 1966[a]	Klamath Lake, Oreg.	24
Cimex lectularius	12[d]	Wild[f]	Jan-Feb 2005	Lupata, Macha, Zambia	
Cimex pilosellus	7[e]	Museum[f]	July 1994	Pend-d'Oreille Valley, British Columbia, Canada	
Cimex pipistrelli	7[e]	Museum[g]	Before 1966[a]	Lab colony derived from England	24
Cimex stadleri	6[e]	Museum[g]	June 1956	Bmo, Czechoslovakia[a]	24
Haematosiphon inodorus	2[e]	Museum[f]	July 1976	Presidio County, Tex.	
Hesperocimex cochimiensis	3[e]	Museum[g]	July 1957	Baja California, Mexico	
Hesperocimex coloradensis	8[e]	Museum[f]	July 1971	Los Alamos County, N.Mex.	
Hesperocimex sonorensis	8[e]	Museum[g]	January 1958	Lab colony of unknown origin	
Hesperoctenes eumops	1[e]	Museum[g]	June 1945	Fresno County, Calif.	
Hesperoctenes fumarius	2[e]	Museum[f]	August 2003	St. John, U.S. Virgin Islands	
Latrocimex sp.	4[e]	Museum[g]	October 1957	Trinidad	
Leptocimex boueti	1[e]	Museum[g]	August 1962	Ivory Coast	
Leptocimex duplicatus	1[e]	Museum[g]	Before 1966[a]	Lab colony derived from Egypt	
Loxapsis malayensis	6[e]	Museum[g]	December 1962	Tasik Bera, Pahang, Malaysia	
Oeciacus hirudinis	19[e]	Museum[g]	July 1960	Faraya, Lebanon	
Oeciacus vicarius	1[e]	Museum[f]	July 2004	Oconee County, S.C.	
Ornithocoris furnarii	9[e]	Museum[g]	January 1958	Lab colony of unknown origin	
Ornithocoris pallidus	1[e]	Museum[g]	1969	Hancock County, Miss	
Ornithocoris toledoi	5[e]	Museum[g]	1957	Ponte Nova, Brazil	
Paracimex borneensis	10[e]	Museum[g]	November 1966	Fraser's Hill, Malaysia	
Paracimex caledoniae	5[e]	Museum[g]	March 1945	New Caledonia	
Paracimex capitatus	11[e]	Museum[g]	July 1966	Edie Creek, Papua New Guinea	
Paracimex gerdheinrichi	3[e]	Museum[g]	May 1966	Rantepao, Indonesia	
Paracimex inflatus	7[e]	Museum[g]	July 1966	Kavieng, Papua New Guinea	
Paracimex reductus	6[e]	Museum[g]	January 1962	Kebar Valley, New Guinea	
Paracimex setosus	5[e]	Museum[g]	February 1962[a]	Sarangan, Java, Indonesia	24[c]
Primicimex cavernis	1[e]	Museum[g]	March 1967	Lake Patzaquaro, Mexico	
Psitticimex uritui	1[e]	Museum[f]	February 1990	La Pampa, Argentina	
Stricticimex transversus	4[e]	Museum[g]	October 1957	Kanye, Botswana	

[a] Data lacking on collection label; information inferred from other sources, if possible.
[b] Data are based on additional information in reference(s) indicated.
[c] Collection date illegible on label; correct date located in reference 24.
[d] Specimens dessicated.
[e] Specimens in ethanol.
[f] Private donor.
[g] Usinger Collection, Essig Museum of Entomology, University of California, Berkeley, Calif.

museum collections can act as a valuable untapped resource for molecular surveys for invertebrate endosymbionts.

MATERIALS AND METHODS

Insect samples. Assayed specimens and collection information are listed in Table 1. Wild specimens were collected from vertebrate hosts or from dwellings, placed into either 100% ethanol (*Afrocimex constrictus*) or dried with silica desiccant (*Cimex lectularius*), and transported to the Johns Hopkins Bloomberg School of Public Health for further processing. Museum specimens came either from private donors or from the Cimicidae collection compiled by Robert Leslie Usinger, a collection of over 10,000 cimicid specimens stored in ethanol and housed at the Essig Museum of Entomology, University of California, Berkeley. The Usinger collection specimens date from 1966 or earlier. All museum specimens had been stored in 95 to 100% ethanol since their collection date (Table 1) and were processed in a manner similar to that used for ethanol-preserved wild material.

DNA extraction. In most cases, we were constrained by the specimen donor in terms of the number of samples that could be processed for DNA extraction. Sample sizes are listed in Table 1. To preserve the external morphology of processed insects and, thus, their value as museum specimens, we used a minimally destructive method for DNA extraction. We tested two variants of the extraction protocol, one based on DNeasy spin columns (QIAGEN, Valencia, CA) and the other based on high salt-ethanol extraction (16). For both protocols, insect abdomens were cut with a sterile razor or punctured several times with a fine needle (for small specimens). For the QIAGEN protocol, specimens were digested overnight (~18 h) in 180 μl 1× phosphate-buffered saline, 20 μl proteinase K, and 200 μl AL buffer solution. The digestate was vortexed with 200 ml of 100% cold ethanol, applied onto DNeasy columns, and DNA bound, washed, and eluted according to the manufacturer's suggested protocol. For the salt extraction protocol, specimens were digested in extraction buffer for ~18 h, and the digestate was processed as described previously (16). After the digestions, the exoskeletons were removed, placed in 100% ethanol, and archived at −20°C. Some specimens were mounted permanently on glass slides using Euparal per-

TABLE 2. *Wolbachia*-specific screening primers used in this study

Primer	Sequence (5′–3′)	Reference
99F	TTG TAG CCT GCT ATG GTA TAA CT	14
994R	GAA TAG GTA TGA TTT TCA TGT	14
WSpecF	CAT ACC TAT TCG AAG GGA TAG	28
WSpecR	AGC TTC GAG TGA AAC CAA TTC	28
INTF1	ACC CTC ATC CTT AGT TGC CAT	This study
INTR1	TGT AGC ACG TGT GTA GCC CAC T	This study
INTF2	AGT CAT CAT GGC CTT TAT GGA	This study
INTR2	TCA TGT ACT CGA GTT GCA GAG T	This study

manent mounting medium (Bioquip Products, Rancho Dominguez, CA). We found that the salt extraction protocol tended to result in higher yields of extracted DNA but observed no differences in PCR success between the two protocols.

***Wolbachia*-specific PCR assays.** All PCRs were conducted using *Cimex lectularius* colony specimens known to be infected as a positive control and a reaction containing all PCR ingredients except template DNA as a negative control. Specimens were assayed individually. Each 25-μl reaction consisted of 1 μl template DNA, 0.4 μM concentrations of all forward and reverse primers, 0.4 mM deoxynucleoside triphosphates, and 2.5 U *Taq* polymerase. Fragments were amplified on a PTC thermocycler (Bio-Rad, Hercules, CA) using a program of 95°C for 5 min; 40 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min; and a final extension of 72°C for 5 min. Fragments were separated by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized by UV light.

PCR was attempted using a variety of published (14, 28) and unpublished (Table 2) primer sets designed to specifically amplify portions of the *Wolbachia* 16S rRNA gene. Due to the wide range of DNA template quality in our samples, different primer combinations were used to amplify fragments ranging from approximately 130 bp to 900 bp. From degraded specimens, the amplification success rate for small fragments (<200 bp) was much greater than that for larger fragments. Primer sequences and amplified fragment arrangements are listed in Table 2 and Fig. 1.

Sequencing. While PCR screening was conducted with multiple specimens per species, sequences were obtained from a single positive specimen of each species. Amplified *Wolbachia* fragments were separated by 1% agarose gel electrophoresis, purified using QIAGEN MinElute columns (QIAGEN), and directly sequenced in both directions using an ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, CA). BioEdit (6) software was used to manually edit sequences.

Phylogenetic analysis. The GenBank database was searched for homologous sequences using the Basic Local Alignment Search Tool (BLAST). Retrieved sequences were aligned with manual correction using BioEdit. Maximum parsimony phylogenetic analyses were conducted using MEGA v. 2.1 (10). Tree support was evaluated by bootstrapping with 500 replications.

Nucleotide sequence accession numbers. The sequences determined in this study were deposited in the GenBank database under accession numbers DQ399339 to DQ399349 and DQ400573.

RESULTS

In total, we assayed 39 Cimicidae species (spanning 16 genera and all 6 recognized subfamilies) (24) and 2 Polyctenidae species for *Wolbachia* infections (Table 1). The *Wolbachia* screening was attempted initially by specific amplification of an approximately 900-bp fragment of the *Wolbachia* 16S rRNA gene using primers 99F and 994R (14). Amplification of this fragment from recently collected wild specimens (*A. constrictus* and *C. lectularius*) generally succeeded but, with the exception of *C. adjunctus*, it was not successful when attempted with museum specimens. Amplification of an approximately 440-bp fragment from the same gene using primers WSpecF and WSpecR (28) produced similar results. We therefore attempted PCR using a set of internal primers (INTF1, INTF2, INTR1, INTR2) within the WSpec amplicon (Fig. 1; Table 2). These primers amplify overlapping fragments ranging from approximately 130 to 240 bp and were designed to be able to sequence the entire WSpec fragment in overlapping amplicons. Because the frequency of contaminant amplification increases with decreasing amplicon size, the 3′ base in each of the short primers was positioned at a synapomorphic site in *Wolbachia* relative to the common strains of background bacteria—*Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp., and *Cowdria* spp.—making the primers *Wolbachia* specific. Not all primer combinations amplified and/or sequenced every specimen, likely due to degraded DNA and/or mutations in the primer binding sites. However, we were able to successfully amplify and confirm by sequencing at least one *Wolbachia*-specific fragment from nine different cimicid species and one polyctenid species (Table 1). Sequences were deposited in the GenBank database. The INTF2-INTR2 primer combination was used for initial screening because it produced a small amplicon and amplified the most consistently. Initial results (not shown) indicated that if the primer pair INTF2-INTR2 did not amplify the expected ~130-bp fragment, other primer combinations never amplified any fragments. Thus, other primer combinations were not tested in later assays if the INTF2-INTR2 PCR failed.

We were able to amplify the entire ~900-bp 99F-994R fragment from infections of *C. lectularius*, *Cimex adjunctus*, and *A. constrictus*. The *C. lectularius* sequence was identical to that previously reported (18). Based on an analysis of 809 bp of the 99F-994R alignment, maximum parsimony analysis supported the inclusion of *C. lectularius* and *C. adjunctus* infections within the F supergroup (bootstrap support, 88%), similar to previously described results for *C. lectularius Wolbachia* infection (18). Analyses indicate inclusion of the *A. constrictus* infection in the A supergroup with weaker support (63%) (Fig. 2).

We were able to amplify the WSpec fragment from the infection of *Cimex hemipterus* by concatenating two internal amplified fragments (Table 1). We also were able to directly amplify the WSpec sequence from the *A. constrictus*, *C. lectularius*, and *C. adjunctus* infections. Based an analysis of a 418-bp WSpec alignment, the *C. hemipterus* infection was included in the F supergroup along with *C. lectularius* and *C. adjunctus*. Bootstrap support for this placement was weaker (64%) due to the smaller size of the nucleotide sequence. The *A. constrictus* infection was once again included in the A supergroup with moderate support (78%) (Fig. 3).

We were not able to amplify either the 99F-994R fragment

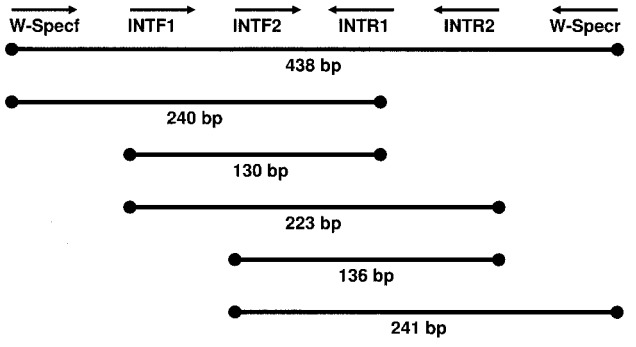

FIG. 1. Amplification schematic and approximate fragment sizes of WSpec and INT *Wolbachia*-specific 16S primers.

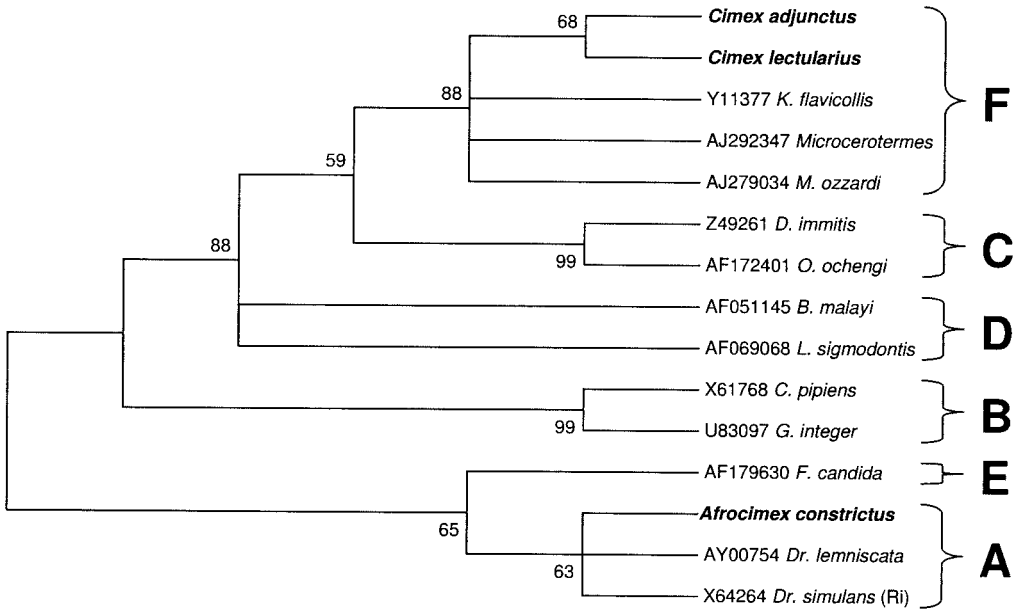

FIG. 2. Maximum parsimony phylogenetic analysis of an 809-bp alignment of *Wolbachia* 16S sequences (99F-994R). Numbers at nodes indicate bootstrap support values (500 replicates). Taxon names are host species. Alphanumeric codes are GenBank accession numbers. Taxa in boldface type indicate cimicid species. Letters represent *Wolbachia* supergroup designations. The tree is unrooted but is presented as midpoint rooted for clarity.

or the entire WSpec fragment from our other specimens. We were, however, able to amplify smaller diagnostic fragments ranging from approximately 130 to 240 bp using various combinations of the internal INT primers. Maximum parsimony analysis of a 241-bp fragment amplified using primer pair INTF2-WSpecR supports the inclusion of *Cimex incrassatus* and *Oeciacus vicarius* in the F supergroup with weak support (54%) due to the small size of the sequence, confirming a previous identification of F *Wolbachia* in *O. vicarius* (18). The infection identified in *Haematosiphon inodorus* was placed in the A supergroup along with *A. constrictus* with relatively weak support due to the small size of the sequence (65%) (Fig. 4).

We were able to amplify, and confirm by sequencing, diagnostic *Wolbachia* fragments from the cimicids *Cimex colum-*

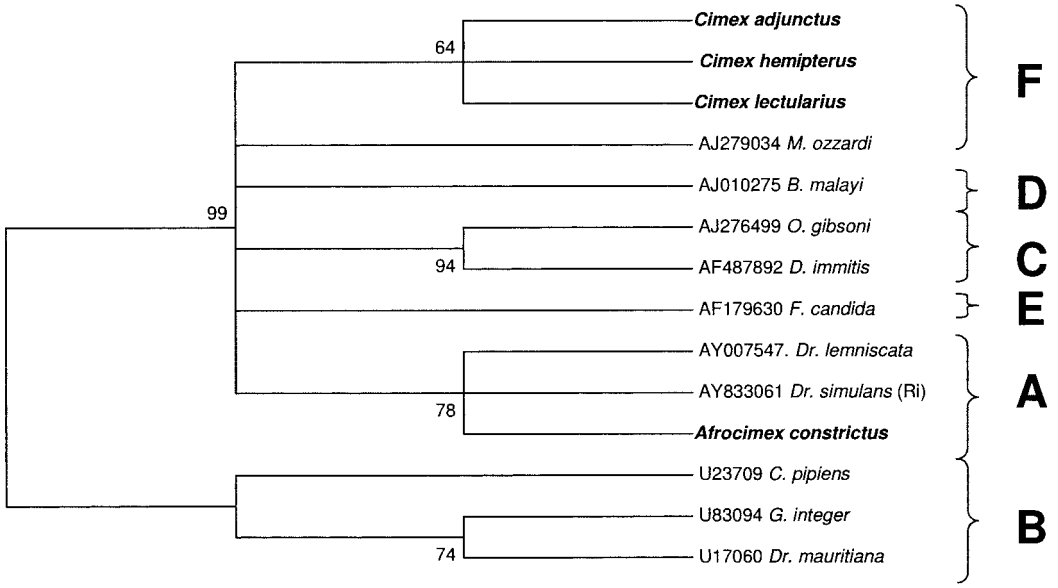

FIG. 3. Maximum parsimony phylogenetic analysis of a 418-bp alignment of *Wolbachia* 16S sequences (WSpecF-WSpecR). Numbers at nodes indicate bootstrap support values (500 replicates). Taxon names are host species. Alphanumeric codes are GenBank accession numbers. Taxa in boldface type indicate cimicid species. Letters represent *Wolbachia* supergroup designations. The tree is unrooted but is presented as midpoint rooted for clarity.

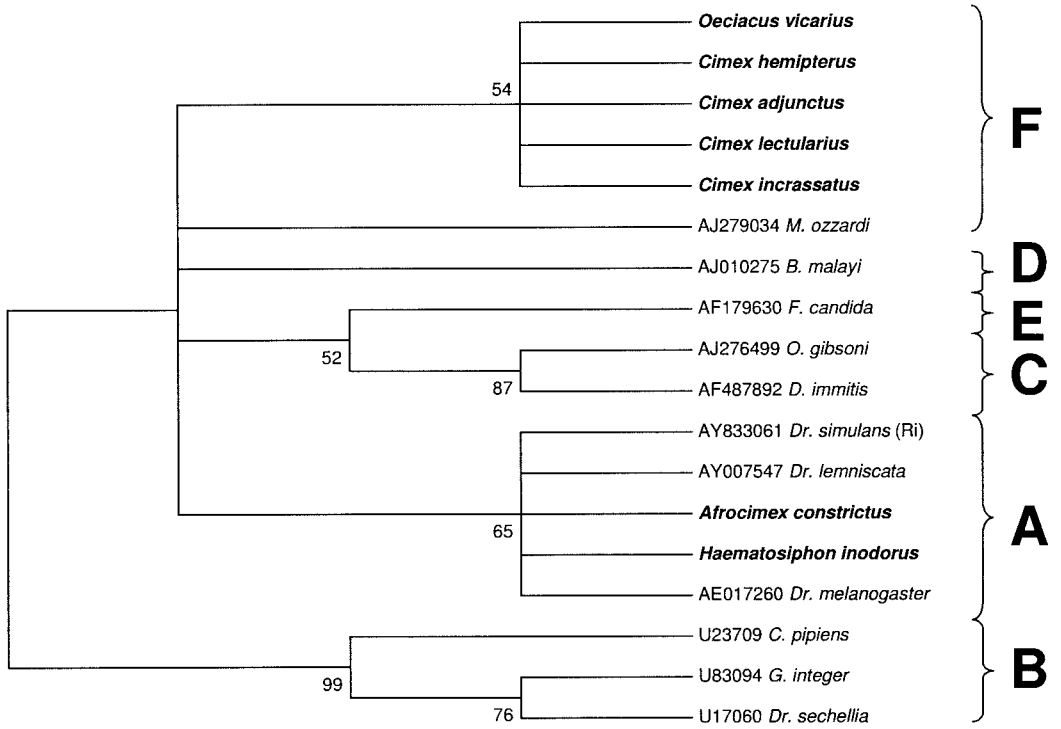

FIG. 4. Maximum parsimony phylogenetic analysis of a 241-bp alignment of *Wolbachia* 16S sequences (INT2F-WSpecR). Numbers at nodes indicate bootstrap support values (500 replicates). Taxon names are host species. Alphanumeric codes are GenBank accession numbers. Taxa in boldface type indicate cimicid species. Letters represent *Wolbachia* supergroup designations. The tree is unrooted but is presented as midpoint rooted for clarity.

barius and *Psitticimex uritui* and from the polyctenid *Hesperoctenes fumarius* (Table 3), but we did not obtain enough sequence information to phylogenetically place these infections into a supergroup.

DISCUSSION

Our survey results suggest that *Wolbachia* infections may be common in the Cimicidae. In this preliminary screen of 39 cimicid species, we identified nine infections, seven of which were newly described. Additionally, we observed *Wolbachia* infection in one of the Polyctenidae (sister family to the Cimicidae). We demonstrated that at least two different *Wolbachia* supergroups infect cimicids. We reconfirmed the presence of F supergroup *Wolbachia* in *C. lectularius* and *O. vicarius* and identified related F supergroup infections in the *Cimex* congenerics *C. hemipterus*, *C. adjunctus*, and *C. incrassatus*. These results suggest that F supergroup infections may be common in the subfamily Cimicinae. We were able to place the *Wolbachia* infections of *A. constrictus* and *H. inodorus* into the A supergroup. *Wolbachia* A supergroup infections are commonly described and infect diverse arthropods (11, 21, 27, 28). It remains to be seen, however, how prevalent *Wolbachia* supergroup A infections are in Cimicidae. We did not obtain sufficient sequence information to confidently phylogenetically place the *Wolbachia* infections of *C. columbarius*, *Psitticimex uritui*, and *Hesperoctenes fumarius*. Definitive phylogenetic placement of these infections is not possible without additional sequence data.

The observation of multiple F supergroup infections among the subfamily Cimicinae is very striking (Table 3). Monophyletic F infections were observed for two genera (*Cimex* and *Oeciacus*), suggesting that in this subfamily, *Wolbachia* was introduced once and has diverged dependently along with the insect hosts. In contrast, A supergroup infections were detected in two widely divergent subfamilies, suggesting multiple introductions of A infections into the Cimicidae. Future surveys to detail the distribution of F and A infections among cimicid and polyctenid species are clearly warranted.

The results presented in this initial survey are almost certainly an underestimate of *Wolbachia* prevalence in cimicids. The failure to detect *Wolbachia* DNA in many species may have been due to true lack of infection, sampling bias due to small sample sizes or, most likely, poor template quality in insufficiently preserved specimens. Many specimens, especially those from the Usinger Collection, were stored without temperature control or ethanol changes for over 40 years. Our results are thus preliminary and should be used to guide future survey efforts using fresh wild-caught material. However, even with poorly preserved material, we observed an infection rate in cimicids of over 23% (9 of 39 species), comparable to other estimates of *Wolbachia* prevalence in arthropod taxa (28).

We have shown in this study that museum specimens can provide a valuable resource for molecular surveys of *Wolbachia* infections, similar to results obtained for other bacterial species. Despite DNA degradation, we were able to amplify diagnostic fragments from ethanol-preserved specimens up to 48 years old. While not all of these fragments were long enough to be phylo-

TABLE 3. *Wolbachia* prevalence and supergroup designations as determined by PCR amplification and sequencing of diagnostic 16S rRNA gene fragments in selected Cimicidae and Polyctenidae[a]

Species	Result with:								*Wolbachia* supergroup
	99F, 994R	WSpecF, WSpecR	INT1F, INT1R	INT2F, INT2R	INT1F, INT2R	WSpecF, INT1R	WSpecF, INT2R	INT2F, WSpecR	
Afrocimicinae									
Afrocimex constrictus	+*	+*							A
Cacodminae									
Aphrania vishnou				−					
Cacodmus vicinus				−					
Leptocimex boueti				−	−				
Leptocimex duplicatus				−					
Loxapsis malayensis				−					
Stricticimex transversus				−					
Cimicinae									
Cimex adjunctus	+*	+*	+	+		−	+	+	F
Cimex antennatus				−	−			−	
Cimex brevis				−	−			−	
Cimex columbarius				+*	−			−	?
Cimex hemipterus				+		+*		+*	F
Cimex incrassatus	−	−	−	+			−	+*	F
Cimex insuetus				−	−			−	
Cimex latipennis				−				−	
Cimex lectularius	+*	+*	+	+*			+	+	F
Cimex pilosellus	−	−	−	−		−	−	−	
Cimex pipistrellis				−				−	
Cimex stadleri				−				−	
Oeciacus hirudinis				−	−				
Oeciacus vicarius	−	−	−	+			+	+*	F
Paracimex borneensis				−					
Paracimex caledoniae				−					
Paracimex capitatus				−					
Paracimex gerdheinrichi				−					
Paracimex inflatus				−					
Paracimex reductus				−					
Paracimex setosus				−					
Haematosiphoninae									
Haematosiphon inodorus	−	−	−	+			−	+*	A
Hesperocimex cochimiensis				−					
Hesperocimex coloradensis	−	−	−	−	−	−	−	−	
Hesperocimex sonorensis				−					
Ornithocoris furnarii				−					
Ornithocoris pallidus	−	−	−	−		−	−	−	
Ornithocoris toledoi				−					
Psitticimex uritui	−	−	+	+	+*	−	−	+	?
Latrocimicinae									
Latrocimex sp.				−					
Primicimicinae									
Bucicimex chilensis				−				−	
Primicimex cavernis				−				−	
Polyctenidae: Hesperocteninae									
Hesperoctenes eumops				−	−				
Hesperoctenes fumarius	−	−	+	+	+*	−	−	+	?

[a] *, sequence obtained; +, *Wolbachia* positive; −, *Wolbachia* negative; ?, unknown supergroup; blank cells, nontested primer combinations. Boldface type indicates *Wolbachia*-positive species. *Wolbachia* supergroup designations are as denoted in Results.

genetically useful, sequencing confirmed that they were all diagnostic for *Wolbachia*. Due to the small sizes of the PCR amplicons, the screening primers listed in this report work well for specimens with less-than-optimum DNA quality and should be useful for other researchers conducting *Wolbachia* surveys.

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