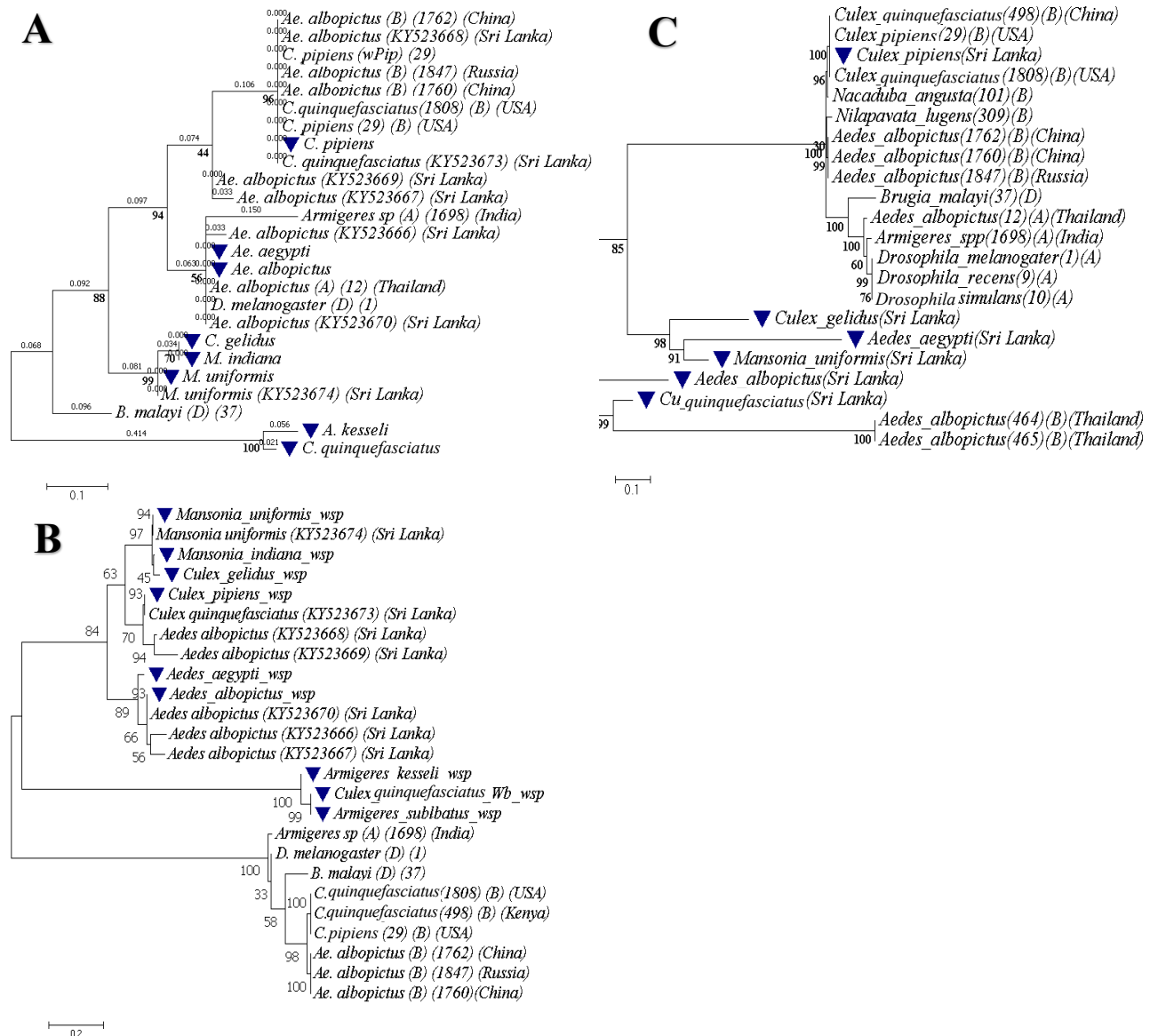


Supplementary Materials

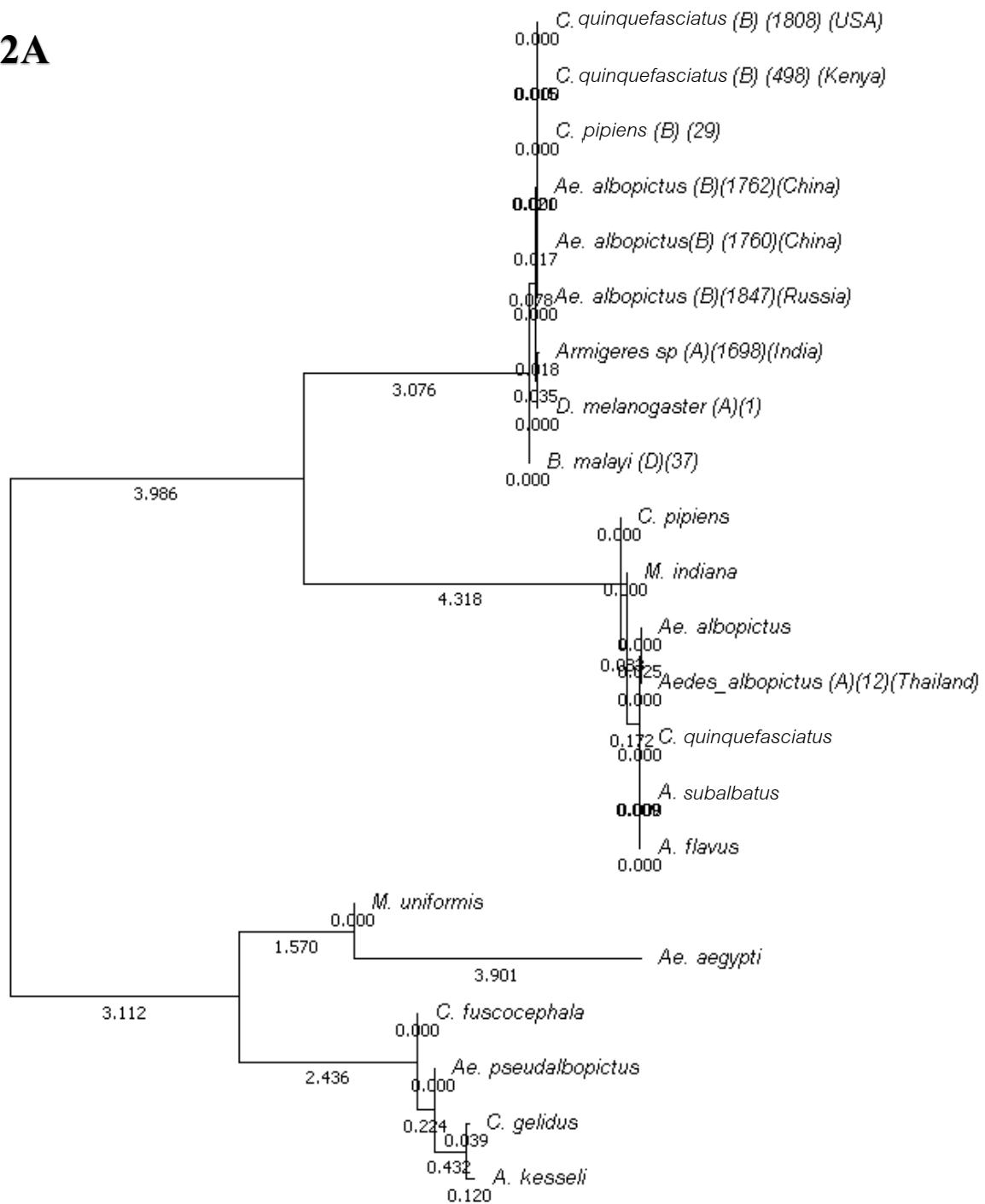
Diversity of *Wolbachia* infections in Sri Lankan mosquitoes with a new record of *Wolbachia* Supergroup B infecting *Aedes aegypti* vector populations

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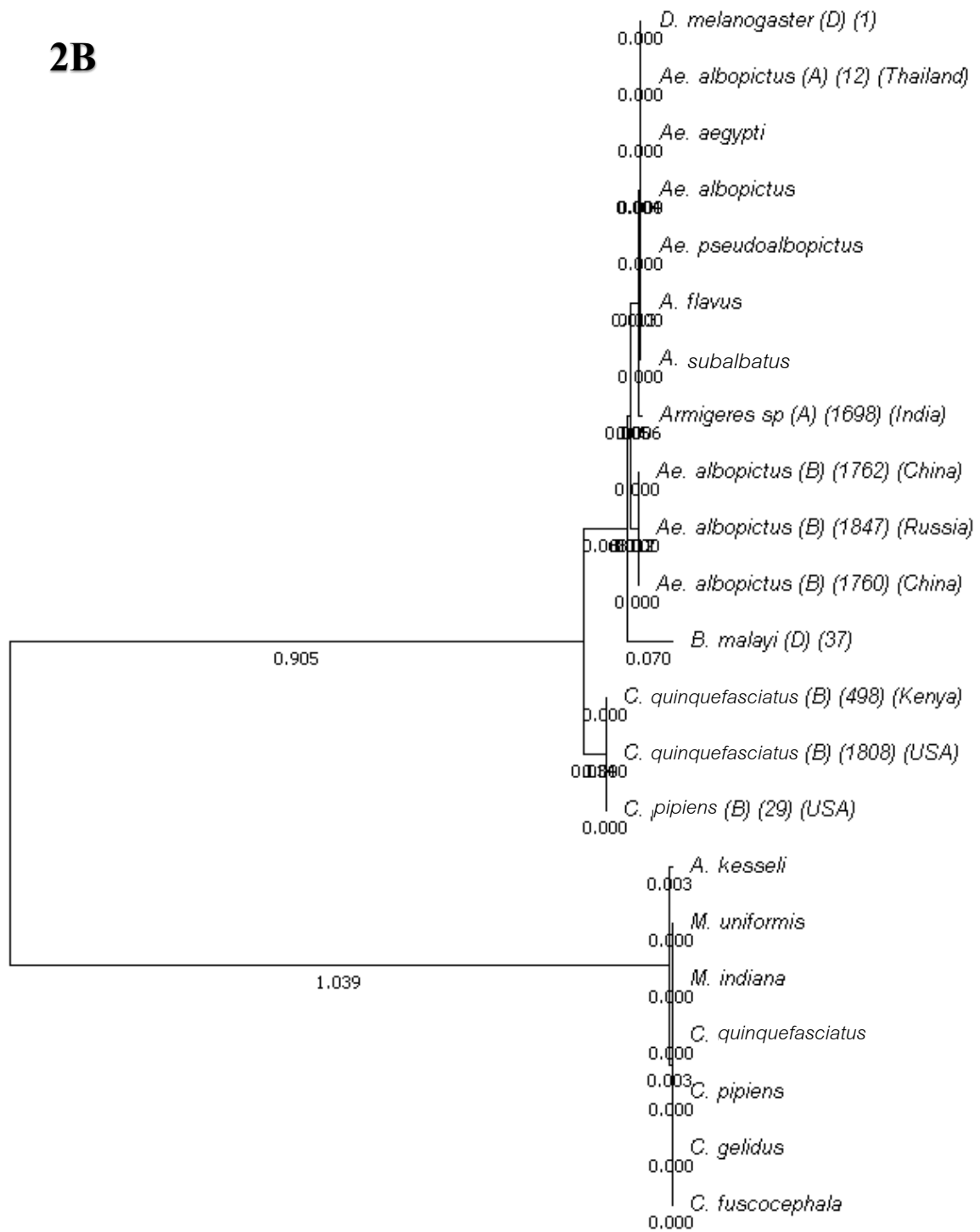
Supplementary Figure 1: *Wolbachia* phylogenetic tree drawn from the sequence data together with reference sequences obtained from the GenBank database. (A) 16S rRNA phylogeny (1 kb), (B) *wsp* phylogeny (590 bp), and (C) phylogeny based on the concatenation of *gatB*, *coxA*, *hcpA*, *ftsZ* and *fbpA* genes (2,074 bp). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The *Wolbachia* D group (allele 37) is used as an out group. *Wolbachia* strains are indicated by their host names and strain names when available. Numbers at the nodes indicate bootstrap values. Only bootstraps higher than 60 are shown. Species are abbreviated as follows; i.e., *Aedes* species: *Ae. aegypti*, *Ae. albopictus*, *Ae. pseudoalbopictus*; *Armigeres* species *Ar. flavus*, *Ar. subalbatus*, *Ar. kesseli*; *Culex* species: *Cx. gelidus*, *Cx. pipiens*, *Cx. quinquefasciatus*; *Mansonia* species: *Mn. indiana*, *Mn. uniformis*; *Brugia malayi*; and *Drosophila melanogaster*.

2A



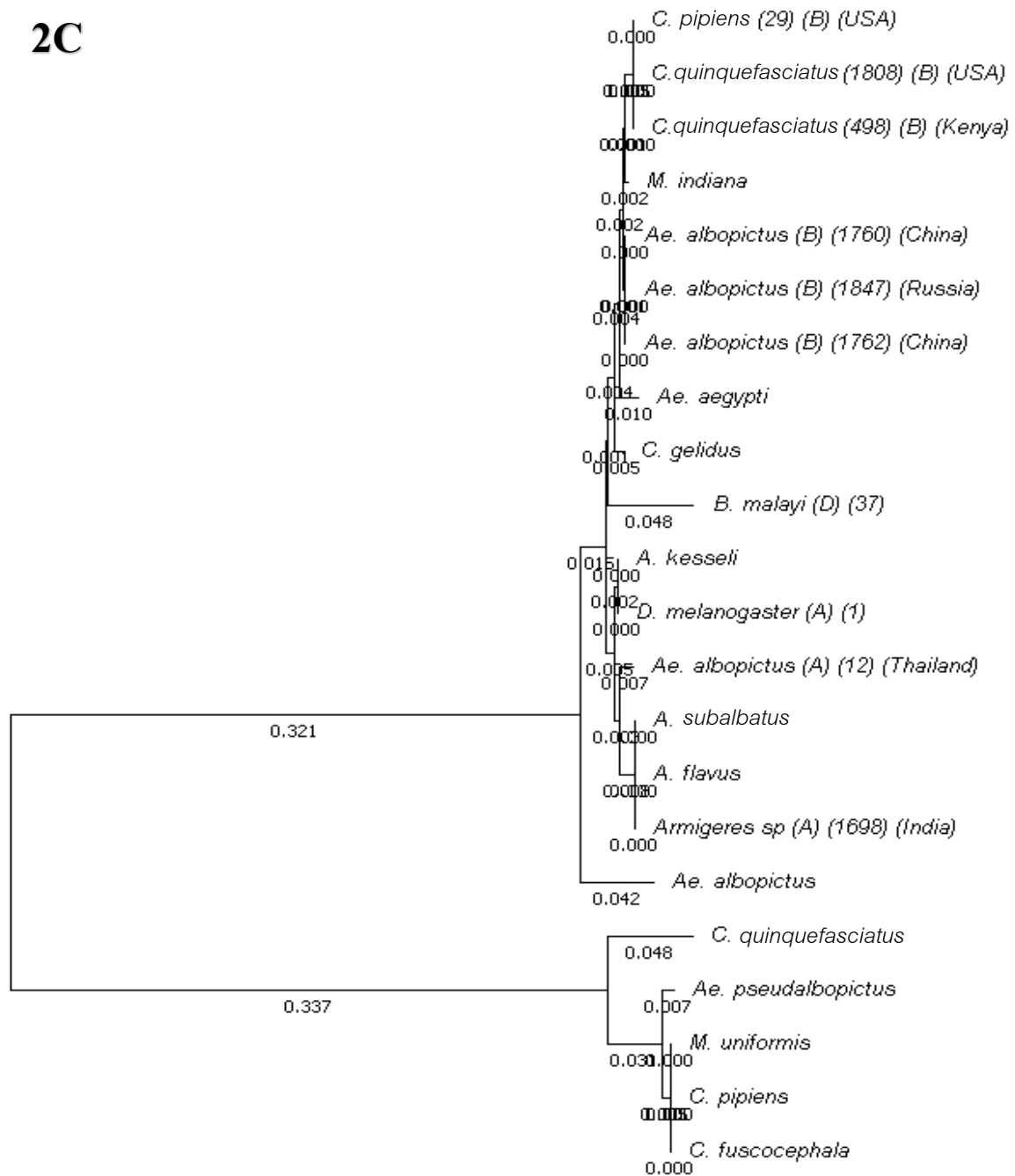
1

2B

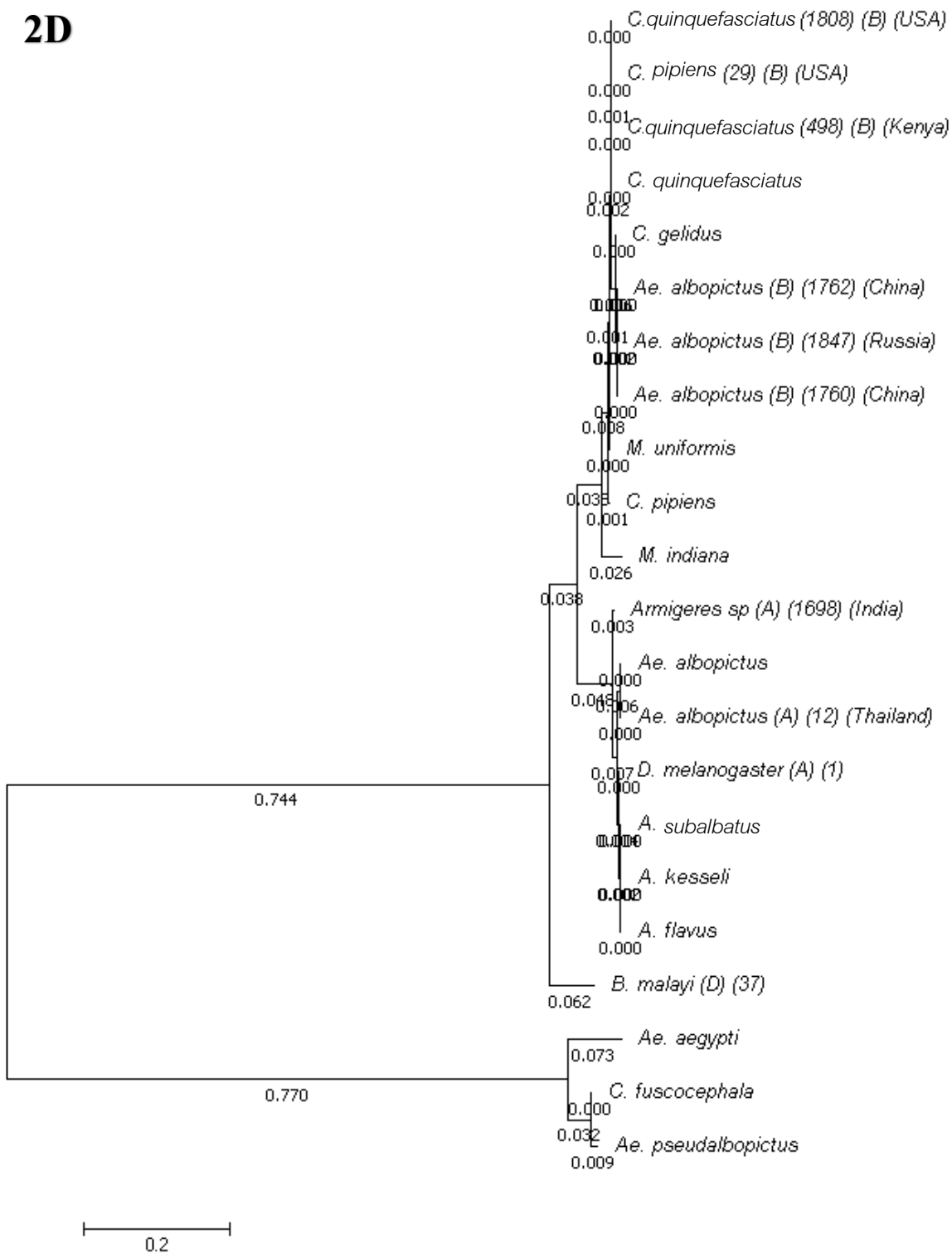


0.2

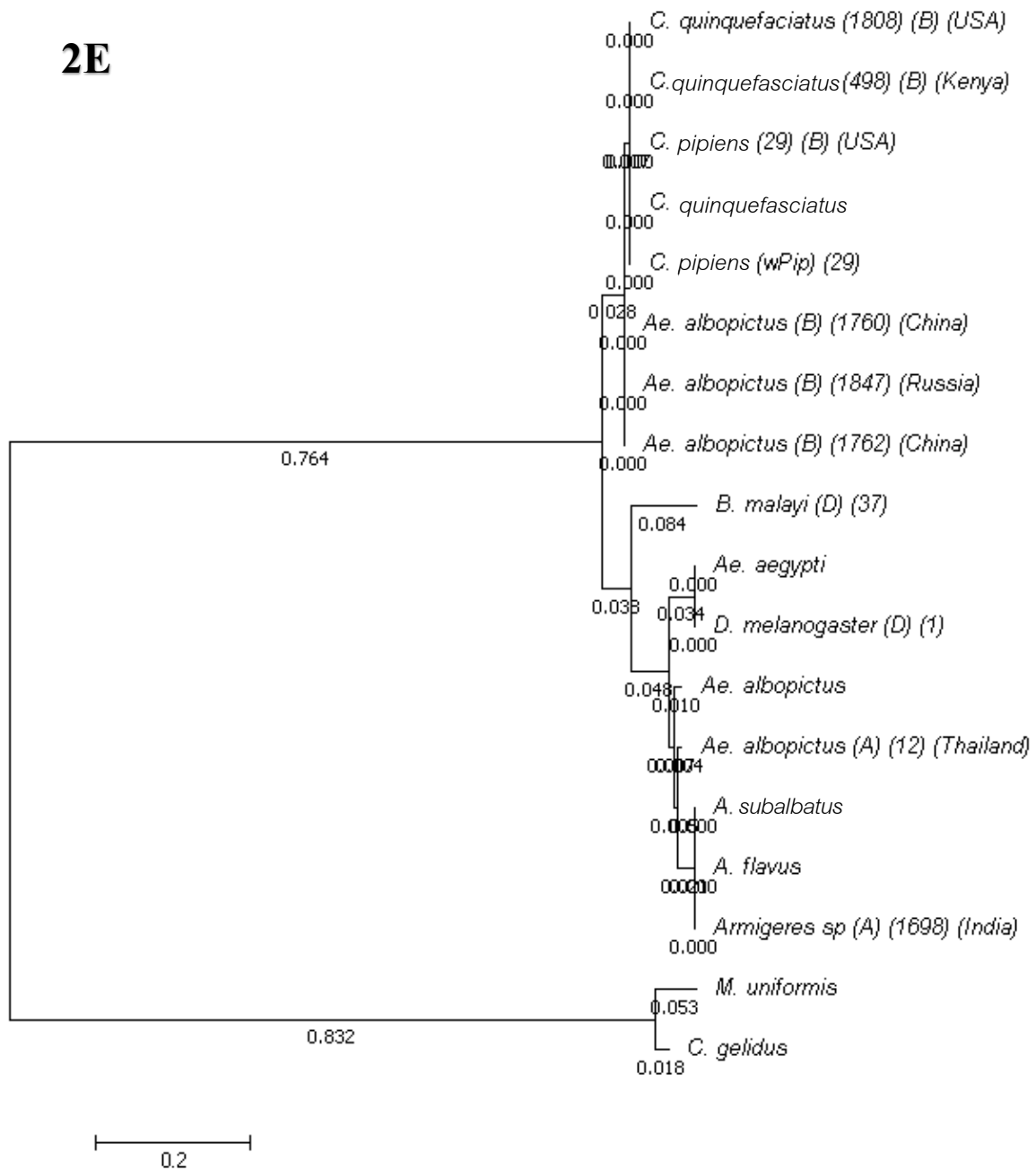
2C



2D



2E



Supplementary Figure 2: Phylogenetic trees analysis of *Wolbachia* strains present in the mosquito species in Sri Lanka, based on different MLST genes: (A) *gatB*, (B) *coxA*, (C) *hcpA*, (D) *ftsZ* and (E) *fbpA*. Numbers at the nodes of each phylogenetic tree indicate bootstrap values. Reference sequences included the host strains from PubMLST.

Supplementary Table S1. Mosquito species screened for *Wolbachia* with *wsp* and *Wolbachia*-specific 16S rRNA primers along with location of collection, collection date, and initial sample size screened.

Genus	Species	Collection location (District)	Collection date (mm/yy)	Sample size
<i>Aedes</i> (16 species)	<i>Aedes (Stegomyia) aegypti</i> Linnaeus, 1762	[¶] Anuradhapura, [¶] Colombo, [¶] Gampaha, Trincomalee	Oct-14 Jun-22 Jun-23	507
	<i>Aedes (Stg) albopictus</i> Skuse, 1894	[¶] Anuradhapura, [¶] Gampaha, [¶] Colombo, [¶] Kandy	Nov-14 Jun-22 Jun-23	21
	<i>Aedes (Finlaya) albotaeniatus</i> Theobald, 1908	Trincomalee	Oct-14	4
	<i>Aedes (Stg) craggi</i> Barraud, 1923	Kandy, Badulla	Nov-14	5
	<i>Aedes (Aedimorphus) jamesi</i> Edwards, 1914	Ampara	Nov-14	4
	<i>Aedes (Stg) krombeini</i> Huang, 1975	Kandy	Nov-14	5
	<i>Aedes (Fin) niveus</i> Ludlow, 1903	Kandy	Nov-14	4
	<i>Aedes (Fin) novoniveus</i> Barraud, 1934	Kandy, Badulla	Nov-14	4
	<i>Aedes (Aed) pallidostriatus</i> Theobald, 1907	Hambanthota, Matara	Dec-13	3
	<i>Aedes (Stg) perplexus</i> Leicester, 1908	Kandy, Badulla	Dec-13	5
	<i>Aedes (Stg) pseudalbopictus</i> Borel, 1928	Kandy, Badulla	Nov-14	6
	<i>Aedes (Cancraedes) simplex</i> Theobald, 1903	Gampaha, Kandy	Mar-14	3
	<i>Aedes</i> spp.	Hambanthota	Dec-13	2
	<i>Aedes (Verrallina) uniformis</i> Theobald, 1910	Anuradhapura, Jaffna, Trincomalee	Sep-13 Jun-22 Jun-23	12
	<i>Aedes (Stg) vittatus</i> Bigot, 1861	Kandy	Dec-13	5
	<i>Aedes (Aed) vexans</i> Meigen, 1830	Hambanthota	Dec-13	5
<i>Anopheles</i> (24 species)	<i>Anopheles (Cellia) aconitus</i> Donitz, 1902	Anuradhapura, Trincomalee, Killinochchi	Feb-14 Jun-22 Jun-23	8
	<i>Anopheles (Anopheles) aitkenii</i> James, 1903	Anuradhapura, Kandy	Oct-14 Jun-22 Jun-23	10
	<i>Anopheles (Cel) annularis</i> Van der Wulp, 1884	Anuradhapura, Trincomalee, Killinochchi	Feb-14 Jun-22 Jun-23	10
	<i>Anopheles (Ano) barbirostris</i> Van der Wulp, 1884	Anuradhapura, Nuwara Eliya, Trincomalee, Mannar, Killinochchi	May-14 Jun-22 Jun-23	10
	<i>Anopheles (Ano) barbumbrosus</i> Strickland & Choudhury, 1927	Anuradhapura, Trincomalee, Mannar	Nov-13 Jun-22 Jun-23	10

Genus	Species	Collection location (District)	Collection date (mm/yy)	Sample size
	<i>Anopheles (Cel) culicifacies</i> Giles, 1901	Anuradhapura, Kurunegala, Nuwara Eliya, Trincomalee	Sep-14 Jun-22 Jun-23	10
	<i>Anopheles (Cel) elegans</i> James, 1903	Kandy	Oct-14	5
	<i>Anopheles (Ano) gigas refutans</i> Alcock, 1913	Nuwara Eliya, Kandy	Sep-14	5
	<i>Anopheles (Ano) insulaeflorum</i> Swellengrebel & Swellengrebel de Graff, 1919	Nuwara Eliya, Kandy	Aug-14	5
	<i>Anopheles (Ano) interruptus</i> Puri, 1929	Nuwara Eliya, Kandy	Aug-14	5
	<i>Anopheles (Cel) jamsii</i> Theobald, 1901	Anuradhapura, Trincomalee, Mannar, Killinochchi	Mar-14 Jun-22 Jun-23	10
	<i>Anopheles (Cel) jeyporiensis</i> James, 1902	Anuradhapura, Trincomalee	Oct-13 Jun-22 Jun-23	10
	<i>Anopheles (Cel) kawari</i> James, 1903	Trincomalee, Kandy	Feb-14	4
	<i>Anopheles (Cel) latens</i> Sallum & Peyton, 2005	Kandy	Oct-14	4
	<i>Anopheles (Cel) maculatus</i> <i>maculatus</i> Theobald, 1901	Kurunegala, Nuwara Eliya, Kandy	Oct-14	4
	<i>Anopheles (Ano) nigerrimus</i> Giles, 1900	Anuradhapura, Trincomalee, Mannar, Killinochchi	Jan-14 Jun-22 Jun-23	10
	<i>Anopheles (Cel) pallidus</i> Theobald, 1901	Anuradhapura, Trincomalee, Mannar, Killinochchi	Jan-14 Jun-22 Jun-23	10
	<i>Anopheles (Ano) peditaeniatus</i> Leicester, 1908	Trincomalee, Mannar, Killinochchi	Nov-13	5
	<i>Anopheles (Ano) peytoni</i> Kulasekera Harrison & Amerasinghe, 1989	Kandy, Trincomalee	Oct-14	5
	<i>Anopheles (Cel) pseudojamesi</i> Strickland & Chowdhury, 1927	Trincomalee, Mannar, Killinochchi	Feb-14	4
	<i>Anopheles (Cel) subpictus</i> Grassi, 1899	Anuradhapura, Trincomalee, Mannar, Killinochchi	Feb-14 Jun-22 Jun-23	10
	<i>Anopheles (Cel) tessellatus</i> Theobald, 1901	Anuradhapura, Trincomalee, Killinochchi	Nov-13 Jun-22 Jun-23	10
	<i>Anopheles (Cel) vagas vagus</i> Donitz, 1902	Kurunegala, Nuwara Eliya, Trincomalee, Mannar, Killinochchi	Mar-14	5
	<i>Anopheles (Cel) varuna</i> Iyengar, 1924	Anuradhapura, Kurunegala, Kandy, Trincomalee, Mannar, Killinochchi	Mar-14 Jun-22 Jun-23	10
<i>Armigeres</i> (7 species)	<i>Armigeres (Arm) aureolineatus</i> Leicester, 1908	Anuradhapura, Kandy	Nov-14 Jun-22 Jun-23	10

Genus	Species	Collection location (District)	Collection date (mm/yy)	Sample size
<i>Culex</i> (21 species)	<i>Armigeres (Lei) balteatus</i> Macdonald, 1960	Badulla	Oct-14	4
	¶ <i>Armigeres (Lei) flavus</i> Leicester, 1908	¶Anuradhapura, ¶Gampaha	Oct-14 Jun-22 Jun-23	10
	<i>Armigeres kesseli</i> Ramalingam, 1987	Gampaha	Oct-14	5
	<i>Armigeres (Leicesteria) magnus</i> Theobald, 1908	Gampaha	Oct-14	5
	<i>Armigeres (Lei) omissus</i> Edwards, 1914	Gampaha	Oct-14	5
	¶ <i>Armigeres (Armigeres) subalbatus</i> Coquillett, 1898	¶Anuradhapura, ¶Gampaha, ¶Colombo, ¶Galle	Oct-14 Jun-22 Jun-23	10
	<i>Culex (Culiciomyia) bahri</i> Edwards, 1914	Nuwara Eliya, Badulla	Oct-14	4
	<i>Culex (Cux) bitaeniorhynchus</i> Giles, 1901	Gampaha, Colombo	Nov-14	3
	<i>Culex (Eumelanomyia) brevipalpis</i> Giles, 1902	Gampaha	Dec-13	4
	<i>Culex (Lutzia) fuscus</i> Wiedemann, 1820	Kandy	Nov-14	4
	¶ <i>Culex (Cux) fuscocephala</i> Theobald, 1907	¶Kandy, ¶Badulla	Nov-14	5
	¶ <i>Culex (Cux) gelidus</i> Theobald, 1901	¶Trincomalee, ¶Gampaha, ¶Colombo	Dec-13	5
	<i>Culex (Cux) hutchinsoni</i> Barraud, 1924	Nuwara Eliya	Dec-13	4
	<i>Culex (Eum) malayi</i> Leicester, 1908	Matara, Galle	Oct-14	4
	<i>Culex (Cux) mimulus</i> Edwards, 1915	Kandy	Oct-14	3
	¶ <i>Culex (Neoculex) pipiens</i> Linnaeus, 1758	¶Gampaha, ¶Colombo, ¶Galle	Dec-13	5
	<i>Culex (Lasiosiphon) pluvialis</i> Barraud, 1924	Gampaha	Dec-13	4
	<i>Culex (Cux) pseudovishnui</i> Colless, 1957	Kandy, Badulla	Dec-13	5
	¶ <i>Culex (Cux) quinquefasciatus</i> Say, 1823	¶Anuradhapura, ¶Gampaha, ¶Colombo, ¶Galle	Oct-13 Jun-22 Jun-23	10
	<i>Culex (Culex) sinensis</i> Theobald, 1903	Matara, Galle	Mar-14	5
	<i>Culex (Cux) sitiens</i> Weidemann, 1828	Matara, Galle	Oct-14	4
	<i>Culex</i> spp.	Badulla	Mar-14	2
	<i>Culex</i> spp.	Trincomalee	Nov-14	2
	¶ <i>Culex (Cux) tritaeniorhynchus</i> Giles, 1901	¶Anuradhapura, ¶Trincomalee, ¶Hambanthota	Nov-14 Jun-22 Jun-23	10

Genus	Species	Collection location (District)	Collection date (mm/yy)	Sample size
	<i>Culex (Lophoceraomyia) uniformis</i> Theobald, 1905	Matara, Galle	Oct-14	5
	<i>Culex (Cux) vishnui</i> Theobald, 1901	Badulla, Nuwara Eliya	Sep-13	5
	<i>Culex (Cux) whitmorei</i> Giles, 1904	Kandy, Gampaha, Colombo	Oct-14	5
<i>Mansonia</i> (5 species)	<i>Mansonia (Mansonioides) annulifera</i> Theobald, 1901	Anuradhapura, Trincomalee	Dec-13 Jun-22 Jun-23	10
	<i>Mansonia (Mnd) dives</i> Van der Wulp, 1881	Nuwara Eliya, Kegalla	Dec-13	4
	[¶] <i>Mansonia (Mnd) indiana</i> Edwards, 1930	[¶] Anuradhapura, [¶] Trincomalee	Dec-13 Jun-22 Jun-23	10
	<i>Mansonia</i> spp.	Trincomalee	Nov-13	3
	[¶] <i>Mansonia (Mnd) uniformis</i> Theobald, 1901	[¶] Anuradhapura, [¶] Gampaha, [¶] Trincomalee, [¶] Colombo	Oct-14 Jun-22 Jun-23	10
<i>Toxorhynchites</i> (2 species)	<i>Toxorhynchites (Toxorhynchites) minimus</i> Theobald, 1905	Gampaha	Nov-14	5
	<i>Toxorhynchites (Tox) splendens</i> Wiedemann, 1819	Gampaha	Nov-14	5
<i>Tripteroides</i> (3 species)	<i>Tripteroides (Tripteroides) affinis</i> Edwards, 1913	Kandy, Kegalla, Badulla, Nuwara Eliya	Nov-14	5
	<i>Tripteroides (Rachionotomyia) aranoides</i> Theobald, 1901	Kegalla, Badulla, Nuwara Eliya	Nov-14	5
	<i>Tripteroides (Trp) ceylonensis</i> Theobald, 1905	Kegalla, Badulla, Nuwara Eliya	Dec-13	5

[¶] *Wolbachia*-infected species and locations

Supplementary Table S2. PCR primers used for mosquito screening of *Wolbachia* bacterial DNA

Gene	Product	Primer		Expected amplicon size	Reference of the primers
		Designation	Sequence (5'–3')		
<i>CO I</i>	Mitochondrial gene cytochrome oxidase c subunit I	<i>CO I</i> -F	GGATTTGGAAATTGATTAGTTCCTT	735 bp	Chan et al., 2014
		<i>CO I</i> -R	AAAAATTTTAATTCCAGTTGGAACAGC		
<i>CO II</i>	Mitochondrial gene cytochrome oxidase c subunit II	A-tLEU - F	AATATGGCAGATTAGTGCA	690 bp	Liu & Beckenbach, 1992
		A-tLEU - R	GTTTAAGAGACCAGTACTT		
<i>glt</i>	<i>Wolbachia</i> genes encoding citrate synthase	WgltAF1	TAC GAT CCA GGG TTT GTT TCT AC	639 bp	Augustinos et al., 2011;
		WgltARev1	CTC ATT AGC TCC ACC GTG TG		
<i>groEL</i>	Heat-shock protein 60	WgroF1	GGTGAGCAGTTGCAAGAAGC	630 bp	Augustinos et al., 2011; Wiwatanara tanabutr et al., 2009
		WgroRev1	AGATCTTCCATCTTGATTCC		
<i>16S rRNA</i>	Partial small subunit ribosomal DNA	16S-F (<i>WspecF</i>)	AGCTTCGAGTGAAACCAATTC	1 kb	Baldo <i>et al</i> , 2006; O'Neill <i>et al.</i> , 1992; Foster <i>et al.</i> , 2008
		16S-R (<i>WspecR</i>)	GAAGATAATGACGGTACTCAC		
MLST					
<i>coxA</i>	Cytochrome <i>c</i> oxidase subunit I	<i>coxA</i> _F1	TTGGRGCRATYAACTTTATAG	487 bp	Baldo <i>et al</i> , 2006
		<i>coxA</i> _R1	CTAAAGACTTTKACRCCAGT		
<i>fbpA</i>	Fructose-bisphosphate aldolase	<i>fbpA</i> _F1	GCTGCTCCRCTTGGYWTGAT	509 bp	Baldo <i>et al</i> , 2006
		<i>fbpA</i> _R1	CCRCCAGARAAAAYYACTATTC		
<i>ftsZ</i>	Cell division protein	<i>ftsZ</i> _F1	ATYATGGARCATATAAARGATAG	524 bp	Baldo <i>et al</i> , 2006
		<i>ftsZ</i> _R1	TCRAGYAATGGATTGATAT		
<i>gatB</i>	Glutamyl-tRNA (Gln) amidotransferase, subunit B	<i>gatB</i> _F1	GAKTTAAAYCGYGCAGGBGTT	471 bp	Baldo <i>et al</i> , 2006
		<i>gatB</i> _R1	TGGYAAYTCRGGYAAAGATGA		
<i>hcpA</i>	Conserved hypothetical protein	<i>hcpA</i> _F1	GAAATARCAGTTGCTGCAAA	515 bp	Baldo <i>et al</i> , 2006
		<i>hcpA</i> _R1	GAAAGTYRAGCAAGYTCTG		
<i>wsp</i>	Outer surface coat protein gene	<i>wsp</i> _F1	GTCCAATARSTGATGARGAAAC	546 bp	Baldo <i>et al</i> , 2006; Zhou et al., 1998; Armbruster et al., 2003
		<i>wsp</i> _R1	CYGCACCAAYAGYRCTRTAAA		

Supplementary Table S3. Components required for the preparation of PCR master mix.

Reagent	(1 X) sample				
	16S rDNA	wsp	MLST	glt/groEL	CO I/II
Water	12.0 µl	14.4 µl	12.2 µl	14.4 µl	17.2 µl
Taqman buffer (10X)	2.0µl	2.0 µl	2.0 µl	2.0 µl	2.5 µl
MgCl ₂ (25 mM)	2 µl	0.5 µl	1.2 µl	0.5 µl	1.0 µl
dNTP mix (10 mM)	1 µl	0.5 µl	0.4 µl	0.5 µl	0.25 µl
Forward primer (10 mM)	0.5 µl	0.2 µl	1.0 µl	0.2 µl	0.35 µl
Reverse primer (10 mM)	0.5 µl	0.2 µl	1.0 µl	0.2 µl	0.35 µl
<i>Taq</i> DNA polymerase (5U/µl)	1 µl	0.2 µl	0.2 µl	0.2 µl	0.25 µl
DNA sample	1 µl	2 µl	2 µl	2 µl	1 µl
Total	20 µl	20 µl	20 µl	20 µl	25 µl

Supplementary Table S4. Thermal profiles for each primer PCR program.

Parameters	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Final hold
Primer						
<i>CO I / CO II</i>	94°C, 3 min	94°C, 1 min	51°C, 1 min	72°C, 2 min	72°C, 10 min	4°C ≡
		35 cycles				
<i>Glt / groEL</i>	95°C, 3 min	94°C, 1 min	48°C, 1 min	72°C, 1 min	72°C, 10 min	4°C ≡
		30 cycles				
<i>16S rDNA</i>	94°C, 3 min	94°C, 1 min	55°C, 1 min	72°C, 2 min	72°C, 10 min	4°C ≡
		35 cycles				
<i>wsp</i>	95°C, 5 min	95°C, 30 sec	59°C, 30 sec	72°C, 30 sec	72°C, 10 min	4°C ≡
		35 cycles				
<i>MLST</i>	94°C, 2 min	94°C, 30 sec	A*, 45 sec	72°C, 1.5 min	72°C, 10 min	4°C ≡
		36 cycles				

A* Annealing temperature for primer *hcpA* - 53°C, *ftsZ* and *gatB* - 54°C, *coxA* - 55°C, *fbpA* - 59°C