Supplemental Material

**SUPPLEMENTAL MATERIAL for the manuscript**

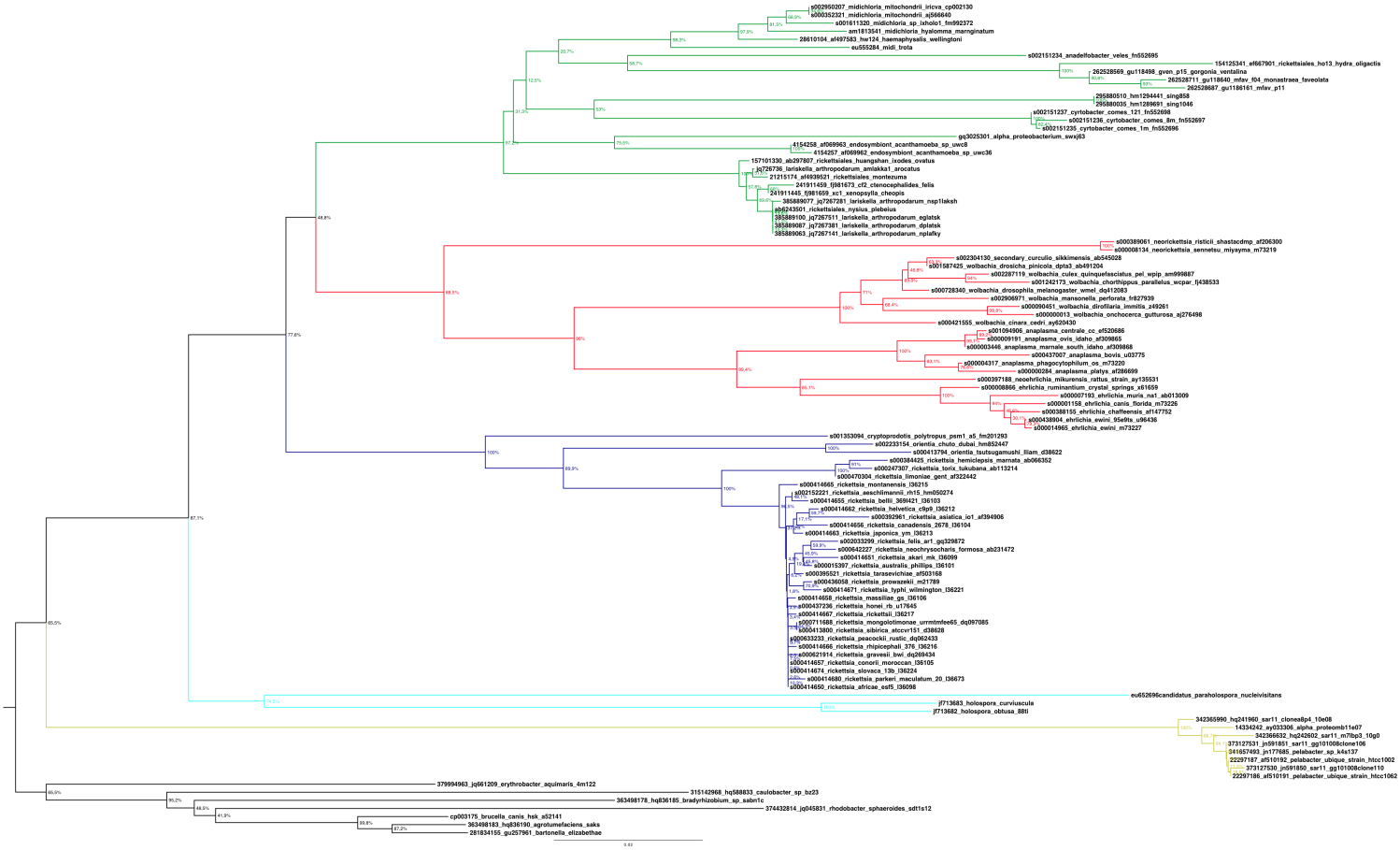
***"Candidatus Midichloriaceae" fam. nov. (Rickettsiales), an ecologically widespread clade of intracellular alpha-proteobacteria***

Submitted to Applied and Environmental Microbiology as Long-Form Papers

This file includes:

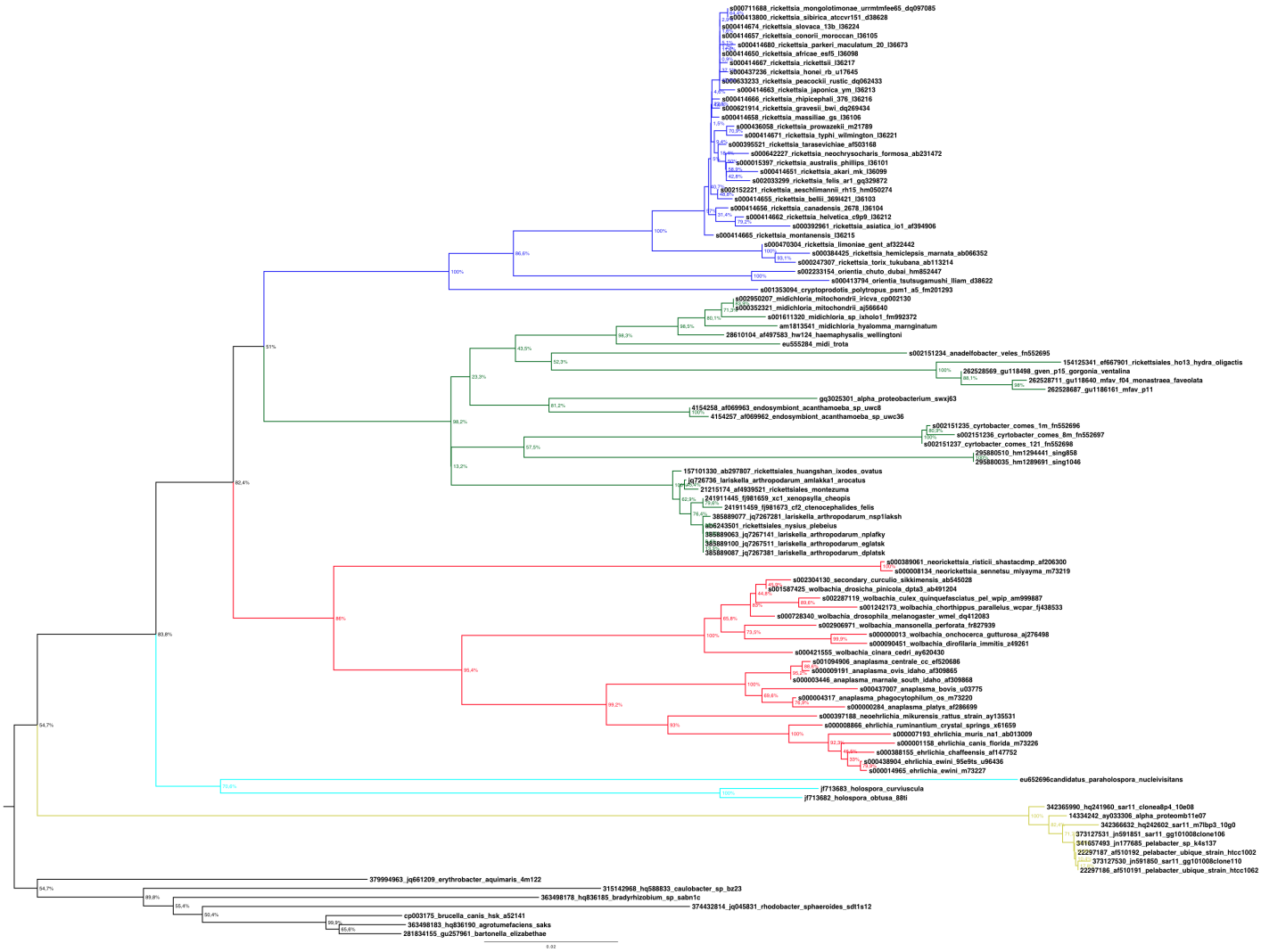
Figures and Legends S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12 and S13.

Table S1.




20FIG. S1. Neighbor-joining phylogram calculated on the 16S rRNA gene sequences Ac
21alignment, bootstrap values are reported on nodes. Branches of the four *Rickettsiales*
22families plus MALOs are reported in different colors: MALOs in green,
23*Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light blue and
24*Pelagibacteraceae* in yellow.


26FIG. S2. Neighbor-joining phylogram calculated on the 16S rRNA gene sequences Al
27alignment, bootstrap values are reported on nodes. Branches of the four *Rickettsiales*
28families plus MALOs are reported in different colors: MALOs in green,
29*Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light blue and
30*Pelagibacteraceae* in yellow.




33FIG. S3. Neighbor-joining phylogram calculated on the 16S rRNA gene sequences Bc

34alignment, bootstrap values are reported on nodes. Branches of the four *Rickettsiales*

35families plus MALOs are reported in different colors: MALOs in green,

36*Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light blue and

37*Pelagibacteraceae* in yellow.


39FIG. S4. Neighbor-joining phylogram calculated on the 16S rRNA gene sequences Bl
40alignment, bootstrap values are reported on nodes. Branches of the four *Rickettsiales*
41families plus MALOs are reported in different colors: MALOs in green,
42*Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light blue and
43*Pelagibacteraceae* in yellow.

44


46FIG. S5. Maximum likelihood phylogram calculated on the 16S rRNA gene

47sequences Ac alignment, bootstrap values are reported on nodes. Branches of the four

48*Rickettsiales* families plus MALOs are reported in different colors: MALOs in green,

49*Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light blue and

50*Pelagibacteraceae* in yellow.

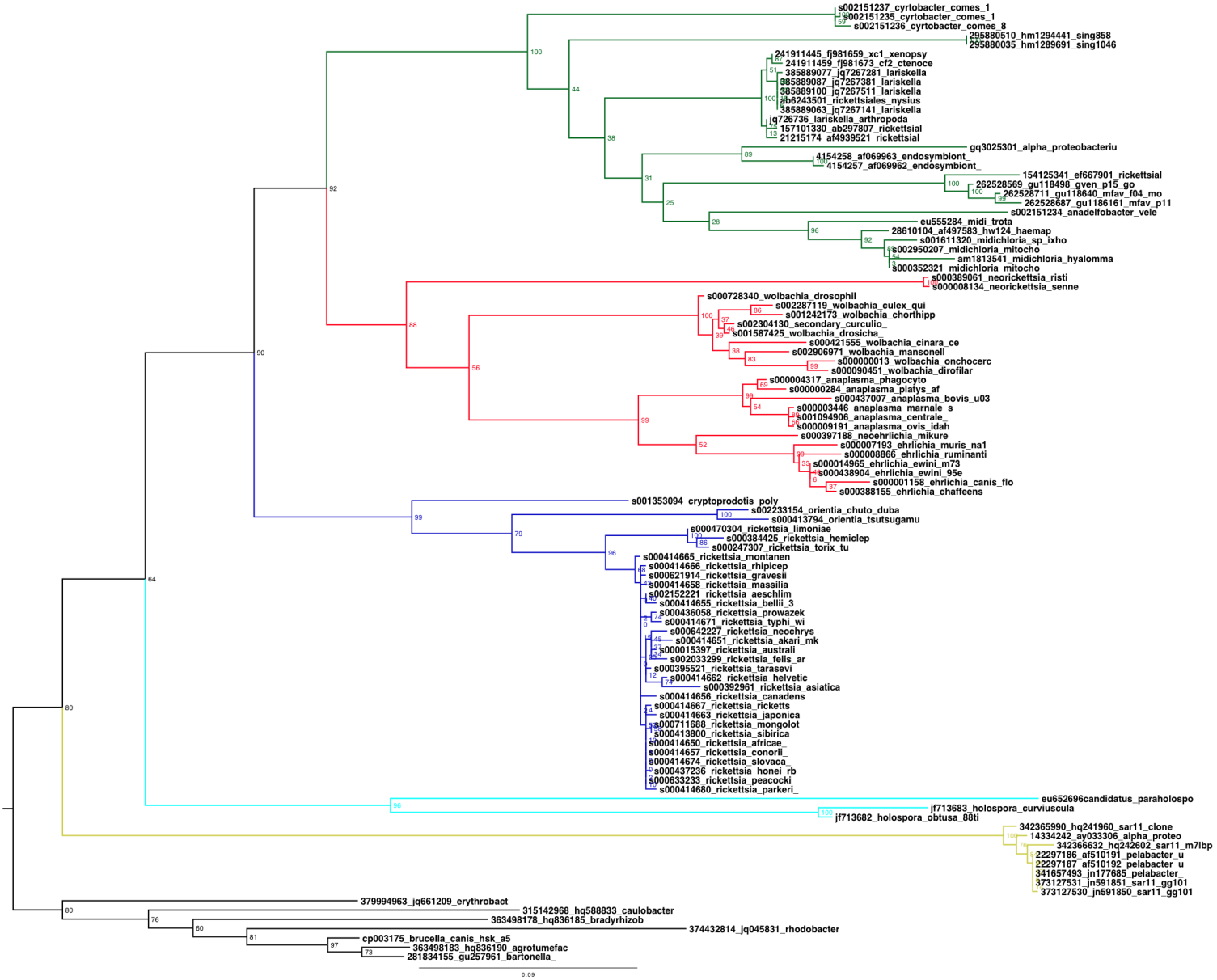

52FIG. S6. Maximum likelihood phylogram calculated on the 16S rRNA gene

53sequences Al alignment, bootstrap values are reported on nodes. Branches of the four

54*Rickettsiales* families plus MALOs are reported in different colors: MALOs in green,

55*Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light blue and

56*Pelagibacteraceae* in yellow.




58FIG. S7. Maximum likelihood phylogram calculated on the 16S rRNA gene

59sequences Bc alignment, bootstrap values are reported on nodes. Branches of the four

60*Rickettsiales* families plus MALOs are reported in different colors: MALOs in green,

61*Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light blue and

62*Pelagibacteraceae* in yellow.

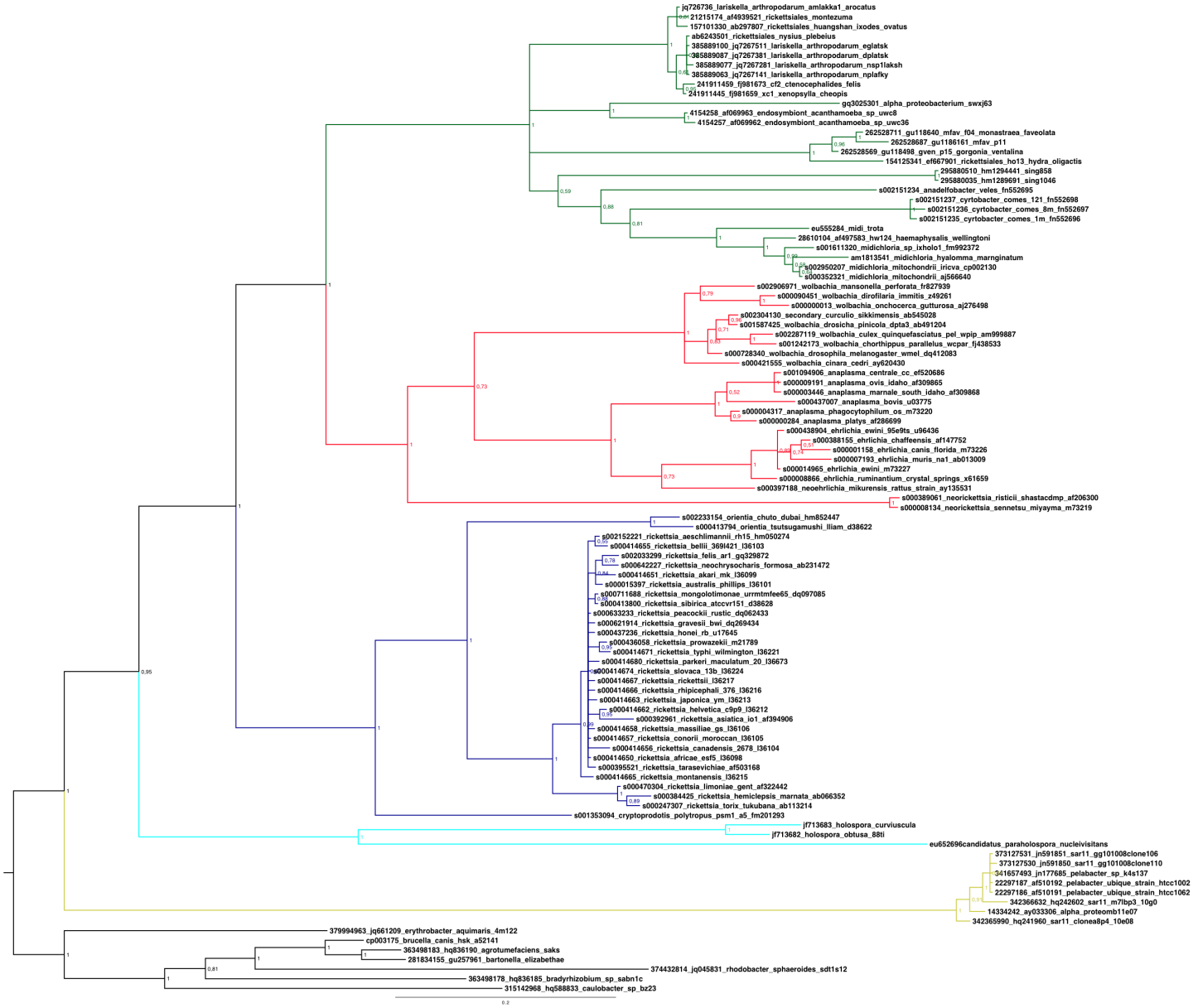

64FIG. S8. Maximum likelihood phylogram calculated on the 16S rRNA gene

65sequences Bl alignment, bootstrap values are reported on nodes. Branches of the four

66*Rickettsiales* families plus MALOs are reported in different colors: MALOs in green,

67*Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light blue and

68*Pelagibacteraceae* in yellow.




70FIG. S9. Bayesian phylogram calculated on the 16S rRNA gene sequences Ac
71alignment, bayesian posterior probability values are reported on nodes. Branches of
72the four *Rickettsiales* families plus MALOs are reported in different colors: MALOs
73in green, *Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light
74blue and *Pelagibacteraceae* in yellow.


76FIG. S10. Bayesian phylogram calculated on the 16S rRNA gene sequences Al

77alignment, bayesian posterior probability values are reported on nodes. Branches of

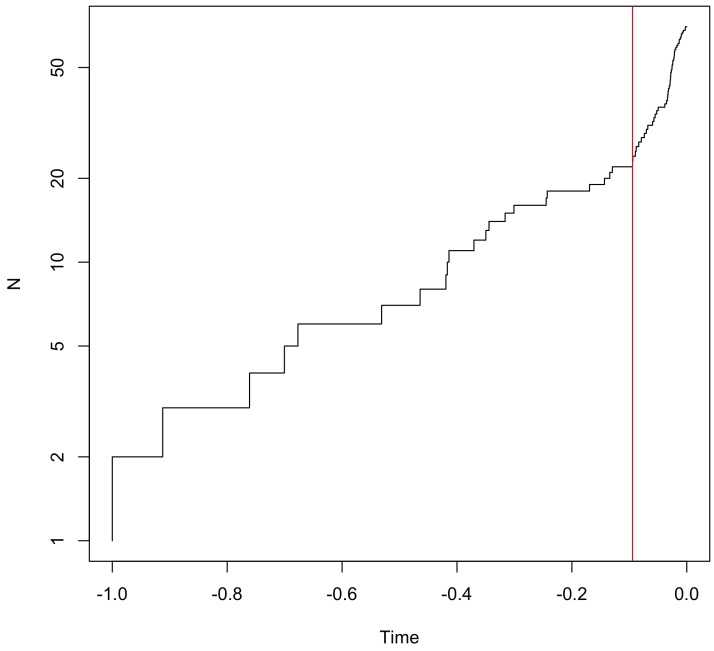
78the four *Rickettsiales* families plus MALOs are reported in different colors: MALOs

79in green, *Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light

80blue and *Pelagibacteraceae* in yellow.


82FIG. S11. Bayesian phylogram calculated on the 16S rRNA gene sequences Bc
83alignment, bayesian posterior probability values are reported on nodes. Branches of
84the four *Rickettsiales* families plus MALOs are reported in different colors: MALOs
85in green, *Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light
86blue and *Pelagibacteraceae* in yellow.


88FIG. S12. Bayesian phylogram calculated on the 16S rRNA gene sequences Bl
89alignment, bayesian posterior probability values are reported on nodes. Branches of
90the four *Rickettsiales* families plus MALOs are reported in different colors: MALOs
91in green, *Anaplasmataceae* in red, *Rickettsiaceae* in dark blue, *Holosporaceae* in light
92blue and *Pelagibacteraceae* in yellow.




93

94FIG. S13. Lineage Through Time Plot.

95

96 Table S1. Accession numbers of 16S rRNA gene sequences

97 belonging to 103 OTUs/species analyzed in this study.

| ORDER | FAMILY | SPECIES | ACCESSION NUMBER |
|---|---|---|---|
| Rhizobiales | Brucellaceae | Brucella canis | CP003175 |
| Rhizobiales | Rhizobiaceae | Agrobacterium tumefaciens | HQ836190 |
| Rhizobiales | Bartonellaceae | Bartonella elizabethae | GU257961 |
| Rhodobacterales | Rhodobacteraceae | Rhodobacter sphaeroides | JQ045831 |
| Rhizobiales | Bradyrhizobiaceae | Bradyrhizobium sp. | HQ836185 |
| Caulobacterales | Caulobacteraceae | Caulobacter sp. | HQ588833 |
| Sphingomonadales | Erythrobacteraceae | Erythrobacter aquimaris | JQ661209 |
| Rickettsiales | Pelagibacteraceae | SAR11 cluster | JN591851 |
| Rickettsiales | Pelagibacteraceae | SAR11 cluster | JN591850 |
| Rickettsiales | Pelagibacteraceae | Pelagibacter sp. | JN177685 |
| Rickettsiales | Pelagibacteraceae | Pelagibacter ubique HTCC1062 | AF510191 |
| Rickettsiales | Pelagibacteraceae | Pelagibacter ubique HTCC1002 | AF510192 |
| Rickettsiales | Pelagibacteraceae | SAR11 cluster | HQ242602 |
| Rickettsiales | Pelagibacteraceae | uncultured MB11E07 (from plankton) | AY033306 |
| Rickettsiales | Pelagibacteraceae | SAR11 cluster | HQ241960 |
| Rickettsiales | Holosporaceae | Holospora curviuscula | JF713683 |
| Rickettsiales | Holosporaceae | Holospora obtusa | JF713682 |
| Rickettsiales | Holosporaceae | Candidatus Paraholospora nucleivisitans | EU652696 |
| Rickettsiales | Rickettsiaceae | Orientia chuto | HM852447 |
| Rickettsiales | Rickettsiaceae | Orientia tsutsugamushi | D38622 |
| Rickettsiales | Rickettsiaceae | Cryptoprodotis polytropus | FM201293 |
| Rickettsiales | Rickettsiaceae | Rickettsia limoniae | AF322442 |
| Rickettsiales | Rickettsiaceae | Rickettsia endosymbiont Hemiclepsis marginata | AB066352 |
| Rickettsiales | Rickettsiaceae | Rickettsia endosymbiont Torix tukubana | AB113214 |
| Rickettsiales | Rickettsiaceae | Rickettsia aeschlimannii | HM050274 |
| Rickettsiales | Rickettsiaceae | Rickettsia rhipicephali | L36216 |
| Rickettsiales | Rickettsiaceae | Rickettsia mongolotimonae | DQ097085 |
| Rickettsiales | Rickettsiaceae | Rickettsia sibirica | D38628 |
| Rickettsiales | Rickettsiaceae | Rickettsia parkeri | L36673 |
| Rickettsiales | Rickettsiaceae | Rickettsia conorii | L36105 |
| Rickettsiales | Rickettsiaceae | Rickettsia africae | L36098 |
| Rickettsiales | Rickettsiaceae | Rickettsia peacockii | DQ062433 |
| Rickettsiales | Rickettsiaceae | Rickettsia rickettsii | L36217 |
| Rickettsiales | Rickettsiaceae | Rickettsia honei | U17645 |
| Rickettsiales | Rickettsiaceae | Rickettsia slovaca | L36224 |
| Rickettsiales | Rickettsiaceae | Rickettsia prowazekii | M21789 |
| Rickettsiales | Rickettsiaceae | Rickettsia typhi | L36221 |
| Rickettsiales | Rickettsiaceae | Rickettsia montanensis | L36215 |
| Rickettsiales | Rickettsiaceae | Rickettsia japonica | L36213 |

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|---|---|---|---|
| Rickettsiales | Rickettsiaceae | Rickettsia helvetica | L36212 |
| Rickettsiales | Rickettsiaceae | Rickettsia asiatica | AF394906 |
| Rickettsiales | Rickettsiaceae | Rickettsia massiliae | L36106 |
| Rickettsiales | Rickettsiaceae | Rickettsia felis | GQ329872 |
| Rickettsiales | Rickettsiaceae | Rickettsia endosymbiont Neochrysocharis formosa | AB231472 |
| Rickettsiales | Rickettsiaceae | Rickettsia akari | L36099 |
| Rickettsiales | Rickettsiaceae | Rickettsia australis | L36101 |
| Rickettsiales | Rickettsiaceae | Rickettsia gravesii | DQ269434 |
| Rickettsiales | Rickettsiaceae | Rickettsia tarasevichiae | AF503168 |
| Rickettsiales | Rickettsiaceae | Rickettsia bellii | L36103 |
| Rickettsiales | Rickettsiaceae | Rickettsia canadensis | L36104 |
| Rickettsiales | Anaplasmataceae | Wolbachia Mansonella perforata | FR827939 |
| Rickettsiales | Anaplasmataceae | Wolbachia Dirofilaria immitis | Z49261 |
| Rickettsiales | Anaplasmataceae | Wolbachia Onchocerca gutturosa | AJ276498 |
| Rickettsiales | Anaplasmataceae | Endosymbiont Curculio sikkimensis | AB545028 |
| Rickettsiales | Anaplasmataceae | Wolbachia Drosicha pinicola | AB491204 |
| Rickettsiales | Anaplasmataceae | Wolbachia Drosophila melanogaster | DQ412083 |
| Rickettsiales | Anaplasmataceae | Wolbachia Culex quinquefasciatus | AM999887 |
| Rickettsiales | Anaplasmataceae | Wolbachia Chorthippus parallelus | FJ438533 |
| Rickettsiales | Anaplasmataceae | Wolbachia Cinara cedri | AY620430 |
| Rickettsiales | Anaplasmataceae | Anaplasma centrale | EF520686 |
| Rickettsiales | Anaplasmataceae | Anaplasma ovis | AF309865 |
| Rickettsiales | Anaplasmataceae | Anaplasma marginale | AF309868 |
| Rickettsiales | Anaplasmataceae | Anaplasma bovis | U03775 |
| Rickettsiales | Anaplasmataceae | Anaplasma phagocytophilum | M73220 |
| Rickettsiales | Anaplasmataceae | Anaplasma platys | AF286699 |
| Rickettsiales | Anaplasmataceae | Ehrlichia ewini | U96436 |
| Rickettsiales | Anaplasmataceae | Ehrlichia ewingii | M73227 |
| Rickettsiales | Anaplasmataceae | Ehrlichia chaffeensis | AF147752 |
| Rickettsiales | Anaplasmataceae | Ehrlichia canis | M73226 |
| Rickettsiales | Anaplasmataceae | Ehrlichia ruminantium | X61659 |
| Rickettsiales | Anaplasmataceae | Ehrlichia muris | AB013009 |
| Rickettsiales | Anaplasmataceae | Neoehrlichia mikurensis | AY135531 |
| Rickettsiales | Anaplasmataceae | Neorickettsia risticii | AF206300 |
| Rickettsiales | Anaplasmataceae | Neorickettsia sennetsu | M73219 |
| Rickettsiales | Midichloriaceae | Cyrtobacter comes | FN552698 |
| Rickettsiales | Midichloriaceae | Cyrtobacter comes | FN552697 |
| Rickettsiales | Midichloriaceae | Cyrtobacter comes | FN552696 |
| Rickettsiales | Midichloriaceae | uncultured bacterium from Kelike Lake | HM129444 |
| Rickettsiales | Midichloriaceae | uncultured bacterium from Kelike Lake | HM128969 |
| Rickettsiales | Midichloriaceae | Lariskella arthropodarum AmLaKka1 | JQ726736 |
| Rickettsiales | Midichloriaceae | Montezuma | AF4939521 |
| Rickettsiales | Midichloriaceae | Huangshan-1 Ixodes ovatus | AB297807 |
| Rickettsiales | Midichloriaceae | Lariskella arthropodarum NpTa1 | AB624350 |
| Rickettsiales | Midichloriaceae | Lariskella arthropodarum NpLaFky | JQ726714 |

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|---|---|---|---|
| Rickettsiales | Midichloriaceae | Lariskella arthropodarum | JQ726751 |
| Rickettsiales | Midichloriaceae | Lariskella arthropodarum | JQ726738 |
| Rickettsiales | Midichloriaceae | Lariskella arthropodarum | JQ726728 |
| Rickettsiales | Midichloriaceae | Uncultured bacterium clone CF2 (from Ctenocephalides felis) | FJ981673 |
| Rickettsiales | Midichloriaceae | Uncultured bacterium clone XC1(from Xenopsylla cheopis) | FJ981659 |
| Rickettsiales | Midichloriaceae | bacterium of Monastraea faveolata | GU118640 |
| Rickettsiales | Midichloriaceae | bacterium of Monastraea faveolata | GU118616 |
| Rickettsiales | Midichloriaceae | bacterium of Gorgonia ventalina | GU118498 |
| Rickettsiales | Midichloriaceae | Uncultured bacterium clone Ho(lakePloen)_13 (from Hydra oligactis) | EF667901 |
| Rickettsiales | Midichloriaceae | uncultured alpha proteobacterium | GQ302530 |
| Rickettsiales | Midichloriaceae | Endosymbiont of Acanthamoeba | AF069963 |
| Rickettsiales | Midichloriaceae | Endosymbiont of Acanthamoeba | AF069962 |
| Rickettsiales | Midichloriaceae | Anadelfobacter veles | FN552695 |
| Rickettsiales | Midichloriaceae | Uncultured bacterium clone ID25L (from Oncorhynchus mykiss) | EU555284 |
| Rickettsiales | Midichloriaceae | uncultured bacterium clone Hw124 (from Haemaphysalis wellingtoni) | AF497583 |
| Rickettsiales | Midichloriaceae | Midichloria sp. Ixholo1 | FM992372 |
| Rickettsiales | Midichloriaceae | Candidatus Midichloria (from Hyalomma marginatum) | AM1813541 |
| Rickettsiales | Midichloriaceae | Midichloria mitochondrii IricVA | CP002130 |
| Rickettsiales | Midichloriaceae | Midichloria mitochondrii | AJ566640 |