**SUPPLEMENTARY FILE 1**

**A dual endosymbiosis supports nutritional adaptation to hematophagy in the invasive tick *Hyalomma marginatum***

Buysse, Floriano *et al.*

**Supplementary files**

**Supplementary File 1a. Summary of the assembly information and quality analyses of the newly sequenced genomes of FLE, *Midichloria* and *Rickettsia* bacteria.** Only genomes with high completeness are shown and the partial genome of *R. aeaschlimannii* (RAES-Hmar-IS) was not presented.

|  |  |  |
| --- | --- | --- |
| **Specimen details** | **Assembling information** |  |
| Genome | Isolate | Origin | Genome size (bp) | Contigs number | N50 | L50 | Completeness (%) | GC rate (%) | Genes | Pseudogenes | Coding density (%) | Coverage values |
| *Francisella-*LE of *Hyalomma marginatum* | FLE-Hmar-ES | Spain | 1510123 | 17 | 254696 | 3 | 0,9905 | 31,11 | 937 | 795 | 54 | 733 |
| *Francisella-*LE of *Hyalomma marginatum* | FLE-Hmar-IT | Italy | 1510130 | 13 | 382079 | 2 | 0,9905 | 31,11 | 934 | 789 | 54 | 950 |
| *Francisella-*LE of *Hyalomma marginatum* | FLE-Hmar-IL | Israel | 1536710 | 25 | 106485 | 5 | 0,9905 | 31,23 | 961 | 798 | 54 | 92 |
| *Midichloria* of *Hyalomma marginatum* | Midi-Hmar-ES | Spain | 1168604 | 179 | 19210 | 20 | 0,981 | 35,03 | 1071 | 192 | 78 | 648 |
| *Midichloria* of *Hyalomma marginatum* | Midi-Hmar-IT | Italy | 1130547 | 359 | 10359 | 30 | 0,9524 | 35,23 | 1032 | 192 | 76 | 80 |
| *Midichloria* of *Hyalomma marginatum* | Midi-Hmar-IL | Israel | 1144281 | 123 | 12627 | 30 | 0,981 | 35,03 | 1055 | 190 | 79 | 63 |
| *Rickettsia aeschlimannii* (of *H. marginatum*) | RAES-Hmar-IT | Italy | 1356794 | 165 | 67640 | 4 | 0,9429 | 32,32 | 1122 | 475 | 68 | 563 |

**Supplementary File 1b. Summary of the information on the NCBI reference genomes employed for the characterization of the FLE and *Midichloria* genomes.**

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| --- | --- | --- | --- | --- | --- | --- |
| **Specimen details** |  |  |  |  | **Assembling information** |  |
| Genome | Isolate | Origin | Accession number (NCBI/dryad) | Reference | Genome size (bp) | Contigs number | GC rate (%) | Coding density (%) |
| *Francisella-*LE of *Ornithodoros moubata* | FLEOm | Lab colony (Czech Republic) | LVCE00000000 | Gerhart et al., 2018 | 1564190 | 8 | 31,7 | 58 |
| *Francisella-*LE of *Ornithodoros moubata* | FOm  | Lab colony (France) | QAPC00000000 | Duron et al., 2018 | 1566493 | 11 | 31,7 | 57 |
| *Francisella-*LE of *Amblyomma maculatum* | FLEAm | United States of America | LNCT00000000 | Gerhart et al., 2018 | 1556261 | 7 | 31,67 | 57 |
| *Francisella-*LE of *Argas arboreus* | *F. persica* (ATCC VR-331) | Egypt | CP013022 | Larson et al., 2016 | 1540768 | 1 | 31,43 | 69 |
| *Francisella-*LE of *Hyalomma asiaticum* | NMGha432 | China | doi.org/10.5061/dryad.t76hdr80p | Buysse and Duron, 2021 | 1550715 | 38 | 31,62 | 58 |
| *Francisella-*LE of *Hyalomma asiaticum* | XJHA498 | China | doi.org/10.5061/dryad.t76hdr80p | Buysse and Duron, 2021 | 1549389 | 39 | 31,6 | 58 |
| *Francisella tularensis* (tularemia agent) | SCHU-S4 | United States of America | AJ749949 | Larsson et al., 2005 | 1892775 | 1 | 32,26 | 83 |
| *Midichloria* of *Ixodes ricinus* | IricVA | Italy | CP002130 | Sassera et al., 2011 | 1183732 |  1 |  36,55 | 71 |

**Supplementary File 1c. Details regarding symbionts used in phylogenetic analyses of B vitamin biosynthetic pathways and streamlined biotin operon of FLE and *Midichloria*.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Specimen details** |  |  |  |  |  |  |  | **Biotin gene origin** |
| Class | Order | Family | Genus/Species | Strain | Host species | Accession number | Reference | Horizontal transfer  | Ancestral |
| Gammaproteobacteria | Thiotrichales | Francisellaceae | *Francisella*-like | FLE-Hmar-IL | Hard tick, *Hyalomma marginatum* (Israel) | - | This study |  | Lost |
|   |   |   | *Francisella*-like | FLE-Hmar-ES | Hard tick, *Hyalomma marginatum* (Spain) | - | This study |  | Lost |
|   |   |   | *Francisella*-like | FLE-Hmar-IT | Hard tick, *Hyalomma marginatum* (Italy) | - | This study |  | Lost |
|   |   |   | *Francisella*-like | NMGha432 | Hard tick, *Hyalomma asiaticum* (China) | - | Buysse and Duron, 2021 |   | + |
|   |   |   | *Francisella*-like | XJHA498 | Hard tick, *Hyalomma asiaticum* (China) | - | Buysse and Duron, 2021 |   | + |
|   |   |   | *Francisella*-like | FLEAm | Hard tick, *Amblyomma maculatum* | LNCT00000000 | Gerhart et al., 2018 |   | + |
|   |   |   | *Francisella*-like | FLEOm | Soft tick, *Ornithodoros moubata* | LVCE00000000 | Gerhart et al., 2018 |   | + |
|   |   |   | *Francisella*-like | FOm | Soft tick, *Ornithodoros moubata* | QAPC00000000 | Duron et al., 2018 |   | + |
|   |   |   | *Francisella*-like | *F. persica* | Soft tick, *Argas arboreus* | CP013022 | Suitor and Weiss, 1961 |   | + |
|   | Legionellales | Coxiellaceae | *Coxiella*-like | CRt | Hard tick, *Rhipicephalus turanicus* | CP011126 | Gottlieb et al., 2015 |   | + |
|   |   |   | *Coxiella*-like | CRm | Hard tick, *Rhipicephalus microplus* | NSHJ01000000 | Guizzo et al., 2017 |   | + |
|   |   |   | *Coxiella*-like | CLEAA | Hard tick, *Amblyomma americanum* | CP007541 | Smith et al., 2015 |   | + |
|   |   |   | *Coxiella*-like | CeAS-UFV | Hard tick, *Amblyomma sculptum* | CP033868 | Direct submission on GenBank (Vidigal et al.) |   | + |
|   |   |   | *Coxiella burnetii* | RSA493 | - | AE016828 | Seshadri et al., 2003 |   | + |
|   |   | Legionellaceae | *Legionella polyplacis* | PsAG | Louse, *Polyplax serrata* | CP021497 | Říhová et al., 2017 | + |   |
|   |   |   | *Legionella pneumophila* | Lens | - | CR628337 | Cazalet et al., 2004 |   | + |
|   | Enterobacterales | Morganellaceae | *Arsenophonus* endosymbiont | CB | Deer ked, *Lipoptena fortisetosa* | CP013920 | Direct submission on GenBank (Novakova et al.) |   | + |
|   |   |   | *Arsenophonus triatominarum* | ATi | Kissing bug, *Triatoma infestans* | LWMI00000000 | Direct submission on GenBank (Darby) |   | + |
|   |   |   | *Providencia siddallii* | officinalis | Leech, *Haementeria officinalis* | CVRF00000000 | Direct submission on GenBank (Manzano-Marin) |   | + |
|   |   | Erwiniaceae | *Buchnera aphidicola* | LSR1 | Aphid, *Acyrthosiphon pisum* | ACFK00000000 | Direct submission on GenBank (Shigenobu et al.) |   | + |
|   |   |   | *Buchnera aphidicola* |  - | Aphid, *Myzus persicae* | MJNC00000000 | Direct submission on GenBank (Mathers et al.) |   | + |
|   |   |   | *Wigglesworthia glossinidia* | Yale colony | Tsetse fly, *Glossina morsitans* | CP003315 | Rio et al., 2012 |   | + |
|   |   |   | *Riesia pediculischaeffi* | PTSK | Louse, *Pediculus schaeffi* | CP012839 | Direct submission on GenBank (Boyd) |   | + |
|   |   | Pectobacteriaceae | *Sodalis*-like endosymbiont | IL | Psyllid, *Bactericera trigonica* | RBZS00000000 | Ghosh et al., 2020 |   | + |
|   |   |   | *Sodalis*-like endosymbiont | Spu | Cicada, *Philaenus spumarius* | NKXM00000000 | Ankrah et al., 2018 |   | + |
|   |   |   |  |   |  |   |   |   |   |
| Alphaproteobacteria | Rickettsiales | Midichloriaceae | *Midichloria* | MID-Hmar-IL | Hard tick, *Hyalomma marginatum* (Israel) | - | This study | + |   |
|   |   |   | *Midichloria* | MID-Hmar-ES | Hard tick, *Hyalomma marginatum* (Spain) | - | This study | + |   |
|   |   |   | *Midichloria* | MID-Hmar-IT | Hard tick, *Hyalomma marginatum* (Italy) | - | This study | + |   |
|   |   |   | *Cand.* Midichloria mitochondrii | IricVA | Hard tick, *Ixodes ricinus* | CP002130 | Sassera et al., 2011 | + |   |
|   |   | Rickettsiaceae | *Rickettsia buchneri* | ISO7 | Hard tick, *Ixodes scapularis* | JFKF00000000 | Kurtti et al., 2015 | + |   |
|   |   |   | *Rickettsia* endosymbiont | REIS | Hard tick, *Ixodes scapularis* | ACLC00000000 | Gillespie et al., 2012 | + |   |
|   |   |   | *Rickettsia felis* | LSU-Lb | Booklouse, *Liposcelis bostrychophila* | JSEL00000000 | Gillespie et al., 2014 |   | + |
|   |   |   | *Rickettsia aeschlimannii* |  - | - | CCER00000000 | Direct submission on GenBank (Urnite platform) |   | + |
|   |   | Anaplasmataceae | *Wolbachia* | wNleu | Bee, *Nomada leucophthalma* | LYUV00000000 | Direct submission on GenBank (Gerth and Bleidorn) | + |   |
|   |   |   | *Wolbachia* | wNfla | Bee, *Nomada flava* | LYUW00000000 | Direct submission on GenBank (Gerth and Bleidorn) | + |   |
|   |   |   | *Wolbachia* | wCle | Bed bug, *Cimex lectularius* | AP013028 | Nikoh et al., 2014 | + |   |
|   |   |   | *Wolbachia* | wStri | Planthopper, *Laodelphax striatellus* | MUIX00000000 | Ju et al., 2020 | + |   |
|   |   |   | *Wolbachia* | wLug | Planthopper, *Nilaparvata lugens* | MUIY00000000 | Ju et al., 2020 | + |   |
|   |   |   | *Wolbachia* | wCfeT | Flea, *Ctenocephalides felis* | CP051156 | Direct submission on GenBank (Driscoll et al.) | + |   |
|   |   |   | *Neorickettsia sennetsu* | Miyayama | - | CP000237 | Dunning Hotopp et al., 2006 | + |   |
|   |   |   | *Neorickettsia risticii* | Illinois | - | CP001431 | Lin et al., 2009 | + |   |
|   |   |   | *Neorickettsia helminthoeca* | Oregon | Salmonid fish | CP007481 | Direct submission on GenBank (Lin et al.) | + |   |
|   |   |   | *Neorickettsia sp.* | 179522 | - | LNGI00000000 | Direct submission on GenBank (Mitreva et al.) | + |   |
|   |   |   | *Ehrlichia ruminantium* | Welgevonden | - | CR767821 | Collins et al., 2005 |   | + |
|   |   |   | *Anaplasma marginale* | Florida | - | CP001079 | Visser et al., 1992 |   | + |
|   |   |   |  |   |   |   |   |   |   |
| Deltaproteobacteria | Desulfovibrionales | Desulfovibrionaceae | *Lawsonia intracellularis* | N343 | - | CP004029 | Sait et al., 2013 | + |   |
|   |   |   | *Lawsonia intracellularis* | PHE/MN1-00 | - | AM180252 | Direct submission on GenBank (Kaur et al.) | + |   |
|   |   |   | *Lawsonia intracellularis* | Fu/JPN | - | QNHO00000000 | Direct submission on GenBank (Nishikawa et al.) | + |   |
|   |   |   |  |   |   |   |   |   |   |
|   | Cytophagales | Amoebophilaceae | *Cardinium* | cSfur | Planthopper, *Sogatella furcifera* | CP022339 | Direct submission on GenBank (Zeng) | + |   |
|   |   |   | *Cardinium* | cEper1 | Wasp, *Encarsia pergandiella* | HE983995 | Penz et al., 2012 | + |   |
|   |   |   | *Cardinium* | cBtQ1 | Whitefly, *Bemisia tabaci* | CBQZ00000000 | Santos-Garcia et al., 2014 | + |   |

**Supplementary File 1d. Illustrative (non-exhaustive) studies investigating the presence of FLE and *Midichloria* symbionts across *Hyalomma* tick species.** +: presence; –: absence; ND: not determined.

|  |  |
| --- | --- |
| **Specimen details** | **Detection details** |
| Tick species | Geographic origin | Symbiont presence | Detection method | Reference |
| FLE | *Midichloria* |
| *Hyalomma*  | Israel  | + | - | PCR | Azagi et al., 2017 |
| *aegyptium* | Anatolia, Mugla Province | + | ND | Metagenomic analysis | Brinkmann et al., 2019 |
|   | Qatar, Doha | ND | + | PCR | Barradas et al., 2020 |
|  | Bulgaria | + | ND | PCR | Ivanov et al., 2011 |
|  |   |  |  |   |   |
| *H. anatolicum* | Turkey  | - | - | PCR | Duron et al., 2017 |
|   | Turkey | ND | + | PCR & qPCR | Cafiso et al., 2016 |
|   | Pakistan, Punjab Province | + | + | Bacterial barcoding | Adegoke et al., 2020 |
|   |   |  |  |   |   |
| *H. asiaticum* | China, Xinjiang Province | + | ND | PCR | Wang et al., 2018 |
|  | China | + | - | Metagenomic analysis | Buysse and Duron, 2021 |
|   |   |  |  |   |   |
| *H. dromedarii* | Israel | + | ND | PCR | Azagi et al., 2017 |
|  | Saudi Arabia | + | ND | PCR | Elbir et al., 2020 |
|  | Tunisia, Sousse | ND | + | PCR | Selmi et al., 2019 |
|  | Tunisia, Kebili | ND | - | PCR | Selmi et al., 2019 |
|  | Egypt | + | ND |   | Ghoneim et al., 2017 |
|  | United Arab Emirates, Al-Ain | + | ND | Bacterial barcoding | Perveen et al., 2020 |
|   | Palestine, Jericho | + | - | Metagenomic analysis | Ravi et al., 2018 |
|   | Saudi Arabia, Al Hofuf | + | - | PCR & bacterial barcoding | Elbir et al., 2019 |
|   | Saudi Arabia, Hail Province | + | - | Bacterial barcoding | Alreshidi et al., 2020 |
|   |   |  |  |   |   |
| *H. excavatum* | Israel | + | ND | PCR | Azagi et al., 2017 |
|   | Tunisia, Kebili | ND | - | PCR | Selmi et al., 2019 |
|   | Turkey, Mecitözu | + | + | PCR | Duron et al., 2017 |
|   | Turkey | ND | + | PCR & qPCR | Cafiso et al., 2016 |
|  | Egypt | ND | + | Reverse line blotting (RLB) | AL-Hosary et al., 2021 |
|   |   |  |  |   |   |
| *H. impeltatum* | Tunisia, Gabes | ND | + | PCR | Selmi et al., 2019 |
|  | Tunisia, Sousse | ND | + | PCR | Selmi et al., 2019 |
|  | Tunisia, Kebili | ND | - | PCR | Selmi et al., 2019 |
|  | Tunisia, Kairouan | ND | - | PCR | Selmi et al., 2019 |
|   | Tunisia, Tataouine | ND | - | PCR | Selmi et al., 2019 |
|   | Zimbabwe | + | - | PCR | Duron et al., 2017 |
|   |   |  |  |   |   |
| *H. lusitanicum*  | Spain | + | + | Bacterial barcoding | Díaz-Sánchez et al., 2021 |
|  | Canaries, Veneguera | + | - | PCR | Duron et al., 2017 |
|  | Spain, Madrid | + | ND | PCR | Lopes de Carvalho et al., 2016 |
|  | Portugal | - | ND | PCR | Lopes de Carvalho et al., 2016 |
|   |   |  |  |   |   |
| *H. marginatum*  | Israel  | + | ND | PCR & FISH | Azagi et al., 2017 |
|  | Anatolia, Mugla Province | + | ND | Illumina HiSeq sequencing | Brinkmann et al., 2019 |
|  | France | + | + | PCR | Duron et al., 2017 |
|  | Italy, Sicilia | ND | + | PCR | Epis et al., 2008 |
|  | Ventotene Island (Mediterranean Sea) | ND | + | PCR | Di Lecce et al., 2018 |
|  | Bulgaria | + | ND | PCR | Ivanov et al., 2011 |
|  | Spain, Madrid | - | ND | PCR | Lopes de Carvalho et al., 2016 |
|  | Portugal | - | ND | PCR | Lopes de Carvalho et al., 2016 |
|   |   |  |  |   |   |
| *H. rufipes*  | Israel | + | ND | PCR | Azagi et al., 2017 |
|   | China, Gansu Province | - | + | High-throughput sequencing of small RNAs | Luo et al., 2017 |
|   | Ethiopia, Didessa Valley | + | ND | PCR | Szigeti et al., 2014 |
|   |   |  |  |   |   |
| *H. truncatum*  | Namibia | + | ND | PCR  | Direct submission on GenBank (JF290387) |
|   | Italy, Sicilia | ND | + | PCR  | Epis et al., 2008 |

**Supplementary File 1e. Genes and primers used in this study.** Primers were used for qPCR assays conducted for relative quantification of FLE and *Midichloria*.

1 PCR conditions: primers concentration 150 nM; thermal profile: 95 °C for 3 min, and 40 cycles at 95 °C for 15 sec, 55 °C for 20 sec, 72 °C for 10 sec; 2 PCR conditions: primers concentration 250 nM; thermal profile: 95 °C for 3 min, and 40 cycles at 95 °C for 15 sec, 58 °C for 30 sec; 3 PCR conditions: primers concentration 250 nM; thermal profile: 95 °C for 3 min, and 40 cycles at 95 °C for 15 sec, 58°C for 30 sec.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Hypothetical product**  | **Primers (5'-3')** | **Tm (°C)** | **Fragment size** | **Reference** |
| *Hyalomma marginatum* |   |  |  |  |
| *cal* | Calreticulin | F: TACCTCAAGCTGTTCGACTG | 53 |  F/R: 132 bp1 |  This study |
|  |   | R: GCCCTTGTAGTTGAAGATG |  |   |   |
|   |   |   |  |   |   |
| FLE |   |   |  |   |   |
| *rpoB* | DNA-directed RNA  | F: TGTACCTATTGCTACGCCAG | 58 | F/R: 122 bp2 | This study |
|   | polymerase beta chain | R: GCTTACCAGTACGACCATC |  |   |   |
|   |   |   |  |   |   |
| *Midichloria* |   |   |  |   |   |
| *gyrB* | DNA gyrase B subunit | F: ATTTACATGTCAGGGAGTG | 58 | F/R: 155 bp3 | This study |
|   |   | R: CTACGAGTTATTGCTGCTC |   |   |   |

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