**Table 1:** Localities and report of infection of the studied individuals of the Colombian wood turtle *Rhinoclemmys melanosterna*.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Locality | | Coordinates | | | n(nmic) | *Hepatozoon* sp | *Haemogregarina* sp |
| N | | W |
| Antioquia | | | | | | | |
|  | 3. Caucasiaa | 7.87128 | | -75.327 | 10(0) | 0 | 0 |
|  | 8. Puerto Berrioa | 6.5002 | | -74.399 | 1(0) | 0 | 0 |
|  | 7. Yondóa | 6.8057 | | -74.206 | 4(4) | 1\* | 3 |
| Bolívar | | | | | | | |
|  | 2. Arjonaa | 10.267 | | 75.336 | 12(0) | 0 | 0 |
| Cesar | | | | | | | |
|  | 4. Chimichaguaa | 7.87128 | | -75.327 | 1(0) | 0 | 0 |
|  | 6. Terraplena | 7.88283 | | -73.744 | 7(0) | 0 | 0 |
|  | 5. Loma Corredor a | 8.1358 | | -73.775 | 2(0) | 0 | 0 |
| Cordoba | | | | | | | |
|  | 1. Loricaa | 9.2442 | | -75.864 | 10(0) | 0 | 4 |
| Cundinamarca | | | | | | | |
|  | 9. Bogotá  Unidad de Rescate y Rehabilitación de Animales Silvestres (URRAS)b | 4.6397 | | -74.083 | 13(13) | 0 | 0 |
| Meta | | | | | | | |
|  | 10. Villavicencio  Estación de Biología Tropical Roberto Franco (EBTRF)b | 4.14009 | | -73.634 | 10(7) | 0 | 0 |
| Total | | | | | 70(24) | 1 | 7 |
| Prevalence by genus | | | | | | 1.42 | 10 |
| Overall prevalence of infection | | | 11.42 | | |  |  |

n: total number of samples. nmic: number of samples examined by microscopy. \*: individual infected with *H. simidi* sp. nov. a : Localities wherethe turtles were captured from the wild, b: Animal rescue centers where animals were held in captivity.

**Table 2:** Morphometric measurements of gamonts and host cells of *Hepatozoon simidi* sp. nov. Measurements of *H. fitzsimonsi*, *H. colubri* and *H. rarefaciens* are provided for comparison*.*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | ***Hepatozoon simidi* sp*.* nov**  ***Rhinoclemmys melanosterna (*Geoemydidae*)*** |  | ***Hepatozoon fitzsimonsi***  ***Kinixys belliana***  **(Testudinidae)*a*** |  | ***Hepatozoon colubri***  ***Python reticulatus***  ***Erythrolamprus aesculapii***  **(Squamata, Pythonidae, Colubridae)*b,c.*** |  | ***Hepatozoon rarefaciens***  ***Drymarchon corais***  **(Squamata, Colubridae)*d*** |
| ***Uninfected erythrocytes*** | | |  |  |  |  |  |  |
| *Cell area* |  | 145.96 - 190.2 (158.49 ± 14.16) |  |  |  |  |  |  |
| *Cell length* |  | 15.99 - 18.14 (17.216 ± 0.774) |  |  |  |  |  | 17 |
| *Cell width* |  | 10.33 - 12.80 (11.045 ± 0.789) |  |  |  |  |  | 10 |
| *Nucleus area* |  | 15.64 - 26.99 (20.674 ± 3.875) |  |  |  |  |  |  |
| *Nucleus length* |  | 3.94 - 5.88 (5.046 ± 0.620) |  |  |  |  |  |  |
| *Nucleus width* |  | 4.38 - 5.63 (4.985 ± 0.457) |  |  |  |  |  |  |
| *Cell area* |  | 194.11 - 244.82 (226.7 ± 7.19) |  |  |  |  |  |  |
| *Cell length* |  | 21.16 - 23.24 (22.32 ± 0.76) |  |  |  |  |  |  |
| *Cell width* |  | 11.17 -12.79 (12.04 ± 0.58) |  |  |  |  |  |  |
| *Nucleus area* |  | 22.23 - 25.13 (23.732 ± 1.23) |  |  |  |  |  |  |
| *Nucleus length* |  | 3.62 - 6.98 (4.456 ± 1.42) |  |  |  |  |  |  |
| *Nucleus width* |  | 3.67 - 7.33 (6.031 ± 1.39) |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
| ***Immature gamonts*** |  | n= 26 |  | n=12 |  |  |  |  |
| *Host cell-parasite complex* |  |  |  |  |  |  |  |  |
| *Area* |  | 194.8 -291.0 (229.72±10.82) |  |  |  |  |  |  |
| *Length* |  | 19.59- 24.97 (21.77±1.57) |  |  |  |  |  |  |
| *Width* |  | 10.70 - 16.27 (12.43±1.81) |  |  |  |  |  |  |
| *Parasite* |  |  |  |  |  |  |  |  |
| *Parasite area* |  | 79.08 -126.08 (104.02± 11.63) |  |  |  |  |  |  |
| *Parasite length* |  | 15.17 -19.20 (16.97 ± 1.08) |  | 14.3–19.6 (17.8 ± 1.2) |  | 5-6b |  | 11-22 (15.4) |
| *Parasite width* |  | 6.27 -8.75 (7.11 ± 0.51) |  | 1.6–3.0 (2.3 ± 0.4) |  |  |  | 3-10 (5.5) |
| *Parasite nucleus length* |  | 3.61 -6.99 (5.88 ± 0.75) |  | 1.4–2.8 (2.0 ± 0.4) |  |  |  |  |
| *Parasite nucleus width* |  | 3.61 -6.51 (4.82 ± 0.84) |  | 0.7–1.0 (0.9 ± 0.1) |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
| ***Mature gamonts*** |  | n=34 |  | n=36 |  |  |  |  |
| *Host cell-parasite complex* |  |  |  |  |  |  |  |  |
| *Area* |  | 207.35- 314.43 (262.30±12.85) |  |  |  |  |  |  |
| *Length* |  | 21.13 - 25.75 (23.39±0.56) |  |  |  |  |  |  |
| *Width* |  | 11.73 -14.48 (13.28±0.63) |  |  |  |  |  |  |
| *Parasite* |  |  |  |  |  |  |  |  |
| *Parasite area* |  | 90.83 -129.29 (110.13 ± 7.50) |  |  |  |  |  |  |
| *Parasite length* |  | 15.85 -18.70 (17.42 ± 0.59) |  | 17.1–17.7 (17.5 ± 0.3) |  | 9-9.5b /15-17c |  |  |
| *Parasite width* |  | 6.64 -8.05 (7.25 ± 0.36) |  | 3.3–4.3 (3.9 ± 0.5) |  | 3.8-4.7c |  |  |
| *Parasite nucleus length* |  | 3.05 -5.12 (3.98 ± 0.42) |  | 4.5–5.0 (4.8 ± 0.3) |  |  |  |  |
| *Parasite nucleus width* |  | 4.99 -7.51 (6.04 ± 0.49) |  | 2.4–3.2 (2.9 ± 0.4) |  |  |  |  |

Measurements are given in µm or µm2. Minimum and maximum values and mean ± SD are provided.

a According to (Cook *et al.*, 2009)

bAccording to Börner 1901

cAccording to Han *et al.*, 2015

dAccording to Ball *et al.*, 1967

**Table 3:** 18S rRNA sequences aligned to construct the phylogenetic hypothesis. Sequence length used in each phylogenetic hypothesis of Fig. 3 and Fig. S1 are provided

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Clade | Host | Parasite | GenBank N° |  | Sequence lengths (bp) | | |
|  | Fig. 3A | Fig. S1 A | Fig.S1 B |
| Dactylosomatidae  (Outgroup) | *Pelophylax lessonae (syn. esculentus)* | *Dactylosoma ranarum* | HQ224957 |  | 1808 | 1122 | 580 |
| *Ptychadena anchietae* | *Dactylosoma kermiti* | MN879398 |  | 1737 | 1122 | 580 |
| *Haemogregarina* | *Platysternon megacephalum* | *Haemogregarina pellegrini* | KM887509 |  | 1412 | 1125 | 583 |
| *Mauremys caspica* | *Haemogregarina stepanowi* | KF992697 |  | 1421 | 1124 | 582 |
| *Sacalia quadriocellata* | *Haemogregarina sacaliae* | KM887507 |  | 1418 | 1124 | 582 |
| *Chelydra serpentina* | *Haemogregarina balli* | HQ224959 |  | 1817 | 1126 | 584 |
| *Podocnemis unifilis* | *Haemogregarina* sp. | MW246122 |  | 1423 | 1125 | 583 |
| *Rhinoclemmys melanosterna* | *Haemogregarina* sp*.* RM1 | MT754268 |  | 585 | 585 | 584 |
| *Rhinoclemmys melanosterna* | *Haemogregarina* sp*.* H14 | MT754269 |  | 583 | 583 | 583 |
| *Rhinoclemmys melanosterna* | *Haemogregarina* sp*.* H10 | MT754270 |  | 582 | 582 | 582 |
| *Hepatozoon* | *Lamprophis fuliginosus Boie* | *Hepatozoon ayorgbor* | EF157822 |  | 1773 | 1127 | 585 |
| *Boiga irregularis* | *Hepatozoon boiga* | AF297085 |  | 1996 | 1127 | 589 |
| *Canis lupus familiaris* | *Hepatozoon canis* | MH615006 |  | 1816 | 1124 | 582 |
| *Elaphe carinata* | *Hepatozoon* sp. | KF939620 |  | 1470 | 1088 | 585 |
| *Martes martes* | *Hepatozoon marten* | EF222257 |  | 1757 | 1124 | 582 |
| *Abrothrix olivaceus* | *Hepatozoon* sp. | FJ719817 |  | 1738 | 1127 | 585 |
| *Podarcis bocagei* | *Hepatozoon* sp. | JX531954 |  | 1365 | 1041 | 582 |
| *Caiman crocodilus* | *Hepatozoon* sp. | MW246123 |  | 1394 | 1127 | 585 |
| *Cerdocyon thous* | *Hepatozoon*sp. | KC127679 |  | 1028 | 798 | 600 |
| *Panthera tigris tigris* | *Hepatozoon felis* | HQ829446 |  | 1094 | 831 | 582 |
| *Hemidactylus mabouia* | *Hepatozoon*sp. | KM234615 |  | 1356 | 1029 | 585 |
| *Felis silvestris silvestris* | *Hepatozoon silvestris* | KX757032 |  | 1669 | 1135 | 593 |
| *Sus scrofa leucomystax* | *Hepatozoon apri* | LC314791 |  | 1007 | 930 | 582 |
| *Hepatozoon procyonis* | *Nasua nasua* | MF685409 |  | 1060 | 1015 | 583 |
| *Hyperolius marmoratus* | *Hepatozoon thori* | MG041603 |  | 1640 | 1127 | 585 |
| *Amietia delalandii* | *Hepatozoon theileri* | MG041605 |  | 1673 | 1127 | 585 |
| *Afrixalus fornasini* | *Hepatozoon tenuis* | MG041596 |  | 1701 | 1127 | 585 |
| *Hyperolius marmoratus* | *Hepatozoon involucrum* | MG041591 |  | 1658 | 1127 | 585 |
| *Ctenosaura pectinata* | *Hepatozoon*sp. | MG456821 |  | 1409 | 1089 | 585 |
| *Sauromalus* sp*.* | *Hepatozoon*sp. | MG456822 |  | 1411 | 1089 | 586 |
| *Heloderma horridum* | *Hepatozoon*sp. | MG456823 |  | 1378 | 1089 | 585 |
| *Haemaphysalis bancrofti* | *Hepatozoon**ewingi* | MG593275 |  | 1680 | 1027 | 585 |
| *Gallotia galloti* | *Hepatozoon*sp. | MG787248 |  | 1696 | 1024 | 582 |
| *Tarentola delalandii* | *Hepatozoon*sp. | MG787251 |  | 1698 | 1125 | 582 |
| *Spalerosophis diadema* | *Hepatozoon**aegypti* | MH198742 |  | 1315 | 948 | 469 |
| *Caiman crocodilus* | *Hepatozoon caimani* | MF435048 |  | 1429 | 1065 | 585 |
| *Algyroides marchi* | *Hepatozoon* sp. | JX531944 |  | 1368 | 1041 | 582 |
| *Philodryas nattereri* | *Hepatozoon musa* | KX880079 |  | 1384 | 1021 | 542 |
| *Lithobates (ex. Rana) clamitans* | *Hepatozoon clamatae* | HQ224963 |  | 1655 | 1127 | 585 |
| *Lithobates catesbeianus* | *Hepatozoon catesbianae* | AF130361 |  | 1824 | 1133 | 586 |
| *Amblyomma maculatum* | *Hepatozoon americanum* | AF176836 |  | 1413 | 1140 | 597 |
| *Sclerophrys pusilla* | *Hepatozoon ixoxo* | MG041604 |  | 1631 | 1127 | 585 |
| *Nerodia sipedon sipedon* | *Hepatozoon sipedon* | JN181157 |  | 1807 | 1125 | 585 |
| *Grandisonia alternans* | *Hepatozoon\_seychellensis* | KF246565 |  | 590 | 590 | 585 |
| *Sciurus vulgaris* | *Hepatozoon\_sciuri* | MN104640 |  | 1492 | 1127 | 585 |
| *Philodryas patagoniensis* | *Hepatozoon* sp. | MN003368 |  | 1329 | 944 | 465 |
|  | *Hepatozoon ophisauri* | MN723845 |  | 1721 | 1127 | 585 |
| *Ursus thibetanus japonicus* | *Hepatozoon ursi* | EU041718 |  | 1207 | 1124 | 582 |
| *Panthera pardus pardus* | *Hepatozoon\_luiperdjie* | MN793004 |  | 1002 | 998 | 582 |
| *Zamenis longissimus* | *Hepatozoon colubri* | MN723844 |  | 1609 | 1127 | 585 |
| *Rhinoclemmys melanosterna* | *Hepatozoon simidi* sp*.* nov | MT754271 |  | 584 | 585 | 585 |
| *Hemolivia* | *Rhinella marina* | *Hemolivia stellata* | KP881349 |  | 1816 | 1125 | 583 |
| *Kinixys zombensis* | *Hemolivia parvula* | KR069083 |  | 1052 | 1052 | 582 |
| *Oligoryzomys flavescens* | *Hemolivia* sp. | KU667309 |  | 1051 | 1007 | 585 |
| *Kinixys belliana* | *Hepatozoon fitzsimonsi*a,b | KR069084 |  | 1034 | 1032 | 585 |
| *Testudo graeca* | *Hemoliva mauritanica* | KF992710 |  | 1418 | 1129 | 583 |
| *Egernia stokesii* | *Hemolivia mariae* | KF992712 |  | 1373 | 1124 | 582 |
| *Rhinoclemmys pulcherrima* | *Hemolivia* sp*.* | KF992714 |  | 1421 | 1124 | 582 |
| *Karyolysus* | *Ixodes ricinus* | *Karyolysus lacazei* | MK497254 |  | 1442 | 1124 | 582 |

**Table 4:** Genetic distance calculated using K2P model of substitutions, between 18SrRNA lineages of Adeleorina parasites for the three different alignments in Fig. 3. and Fig. S1.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | | Genetic distance (d ± SD) | | |
| Full lenght sequences  (Fig. 3) | 1000 bp  (Fig. S1 A) | 585 pb  (Fig. S1 B) |
| *Hepatozoon* species from amphibians (Clade A) | | | | |
|  | *Hepatozoon tenuis* vs *Hepatozoon simidi* sp. nov. | 0.059 ± 0.010 | 0.059 ± 0.010 | 0.059 ± 0.010 |
|  | *Hepatozoon theileri* vs *Hepatozoon simidi* sp. nov. | 0.055 ± 0.010 | 0.055 ± 0.010 | 0.055 ± 0.010 |
|  | *Hepatozoon catesbianae* vs *Hepatozoon simidi* sp. nov. | 0.061 ± 0.010 | 0.061 ± 0.010 | 0.059 ± 0.010 |
|  | *Hepatozoon ixoxo* vs *Hepatozoon simidi* sp. nov. | 0.063 ± 0.010 | 0.063 ± 0.010 | 0.063 ± 0.011 |
|  | *Hepatozoon clamatae* vs *Hepatozoon simidi* sp. nov. | 0.065 ± 0.010 | 0.065 ± 0.010 | 0.065 ± 0.011 |
|  | *Hepatozoon involucrum* vs *Hepatozoon simidi* sp. nov. | 0.065 ± 0.010 | 0.065 ± 0.010 | 0.065 ± 0.011 |
|  | *Hepatozoon thori* vs *Hepatozoon simidi* sp. nov. | 0.066 ± 0.010 | 0.066 ± 0.010 | 0.066 ± 0.011 |
| *Hepatozoon* species from reptiles (Clade B) | |  |  |  |
|  | *Hepatozoon* *boiga* vs *Hepatozoon simidi* sp. nov | 0.074 ± 0.012 | 0.076 ± 0.011 | 0.07 ± 0.011 |
|  | *Hepatozoon* *sciuri* vs *Hepatozoon simidi* sp. nov | 0.044 ± 0.009 | 0.044 ± 0.009 | 0.044 ± 0.009 |
|  | *Hepatozoon* *ayorgbor* vs *Hepatozoon simidi* sp. nov | 0.031 ± 0.007 | 0.031 ± 0.007 | 0.031 ± 0.007 |
|  | *Hepatozoon* *seychellensis* vs *Hepatozoon simidi* sp. nov | 0.044 ± 0.009 | 0.044 ± 0.009 | 0.044 ± 0.009 |
|  | *Hepatozoon* *ophisauri* vs *Hepatozoon simidi* sp. nov | 0.031 ± 0.007 | 0.031 ± 0.007 | 0.031 ± 0.007 |
|  | *Hepatozoon caimani* vs *Hepatozoon simidi* sp. nov | 0.037 ± 0.008 | 0.037 ± 0.008 | 0.037 ± 0.008 |
| *Hepatozoon* species from reptiles (Clade C) | |  |  |  |
|  | *Hepatozoon* *colubri* vs *Hepatozoon simidi* sp. nov | 0.019 ± 0.006 | 0.019 ± 0.006 | 0.019 ± 0.006 |
| *Hepatozoon* species from other Testudines | |  |  |  |
|  | *Hepatozoon* *fitzsimonsi* vs *Hepatozoon simidi* sp. nov | 0.030 ± 0.007 | 0.030 ± 0.008 | 0.030 ± 0.007 |
| *Hepatozoon* species from mammals (Clade D) | |  |  |  |
|  | *Hepatozoon felis* vs *Hepatozoon simidi* sp. nov | 0.033 ± 0.007 | 0.033 ± 0.008 | 0.033 ± 0.008 |
|  | *Hepatozoon apri* vs *Hepatozoon simidi* sp. nov | 0.046 ± 0.010 | 0.046 ± 0.009 | 0.046 ± 0.009 |
|  | *Hepatozoon procyonis* vs *Hepatozoon simidi* sp. nov | 0.050 ± 0.010 | 0.050 ± 0.009 | 0.050 ± 0.010 |
|  | *Hepatozoon ursi* vs *Hepatozoon simidi* sp. nov | 0.052 ± 0.009 | 0.052 ± 0.009 | 0.052 ± 0.009 |
|  | *Hepatozoon canis* vs *Hepatozoon simidi* sp. nov | 0.052 ± 0.010 | 0.059 ± 0.010 | 0.059 ± 0.011 |
| *Hepatozoon* species from reptiles (Clade E) | |  |  |  |
|  | *Karyolysus\_lacazei* vs. *Hepatozoon simidi* sp. nov | 0.041 ± 0.008 | 0.041 ± 0.008 | 0.041 ± 0.008 |
|  | *Hepatozoon\_sp* (JX531944) vs. *Hepatozoon simidi* sp. nov | 0.048 ± 0.009 | 0.048 ± 0.009 | 0.048 ± 0.009 |
|  | *Hepatozoon\_sp* (JX787251) vs. *Hepatozoon simidi* sp. nov | 0.071 ± 0.012 | 0.071 ± 0.012 | 0.071 ± 0.011 |
| *Hemolivia* (clade F) | |  |  |  |
|  | *Hemolivia stellata* vs *Hepatozoon simidi* sp. nov | 0.033 ± 0.008 | 0.033 ± 0.007 | 0.033 ± 0.007 |
|  | *Hemolivia párvula* vs *Hepatozoon simidi* sp. nov | 0.035 ± 0.008 | 0.035 ± 0.007 | 0.035 ± 0.008 |
|  | *Hemolivia mariae* vs *Hepatozoon simidi* sp. nov | 0.037 ± 0.008 | 0.037 ± 0.008 | 0.037 ± 0.008 |
|  | *Hemolivia sp in R. pulcherrima* vs *Hepatozoon simidi* sp. nov | 0.037 ± 0.008 | 0.037 ± 0.008 | 0.037 ± 0.008 |
|  | *Hemolivia mauritanica* vs *Hepatozoon simidi* sp. nov | 0.035 ± 0.007 | 0.035 ± 0.007 | 0.035 ± 0.007 |
| *Haemogregarina* sp*.* (clades G, H, I) | |  |  |  |
|  | *Haemogregarina* sp*.* RM1vs *Hepatozoon simidi* sp. nov | 0.052 ± 0.009 | 0.052 ± 0.010 | 0.052 ± 0.010 |
|  | *Haemogregarina balli* vs *Hepatozoon simidi* sp. nov | 0.067 ± 0.011 | 0.067 ± 0.011 | 0.067 ± 0.011 |
|  | *Haemogregarina* sp(MW246122)vs *Hepatozoon simidi* sp. nov | 0.072 ± 0.011 | 0.072 ± 0.012 | 0.070 ± 0.011 |
|  | *Haemogregarina* sp*.* H10vs *Hepatozoon simidi* sp. nov | 0.097 ± 0.013 | 0.097 ± 0.013 | 0.097 ± 0.013 |
|  | *Haemogregarina* sp*.* H10vs *Haemogregarina* (MW246122) | 0.059 ± 0.010 | 0.059 ± 0.011 | 0.059 ± 0.011 |
|  | *Haemogregarina* sp*.* H10vs *Haemogregarina sacaliae* | 0.096 ± 0.013 | 0.096 ± 0.014 | 0.096± 0.013 |
|  | *Haemogregarina* sp*.* H10 vs *Haemogregarina* sp*.* RM1 | 0.086 ± 0.013 | 0.086 ± 0.013 | 0.086 ± 0.013 |
|  | *Haemogregarina* sp*.* RM1 vs *Haemogregarina* (MW246122) | 0.057 ± 0.010 | 0.057 ± 0.011 | 0.057 ± 0.009 |
|  | *Haemogregarina* sp*.* RM1 vs *Haemogregarina sacaliae* | 0.050 ± 0.010 | 0.050 ± 0.009 | 0.050 ± 0.009 |
| *Dactylosoma* (outgroup) | |  |  |  |
|  | *Dactylosoma ranarum* vs *Hepatozoon simidi* sp. nov | 0.069 ± 0.011 | 0.069 ± 0.010 | 0.071 ± 0.010 |