**Supplementary Tables**

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**Supplementary Table 1: Primer details used for PCR amplification of *M. muris* for different genetic regions.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Primer name** | **Sequence (5'-3')** | **Gene region** | **Amplicon size [bp]** | **Reference** |
| Nem\_18S\_F | CGCGAATRGCTCATTACAACAGC | 18S | ~815 | Floyd et al. (2005) |
| Nem\_18S\_R | GGGCGGTATCTGATCGCC |  |  |  |
| Mm527\_F | CATTGGACGAGAACTGGGCTC | 18S | ~1.200 | Newly designed |
| Mm1514\_R | TGGGACACCGTTGKTACATG |  |  |  |
| Nem\_ITS+\_NC5\_F | GTAGGTGAACCTGCGGAAGGATCATT | ITS, 5.8S and ITS2 | ~1.100 | Gasser et al. (1995) |
| Nem\_ITS+\_NC2\_R | TTAGTTTCTTTTCCTCCGCT |  |  |  |
| JB3 (NT-COI\_239\_F) | TTTTTTGGGCATCCTGAGGTTTAT | COI | ~359 | Bowles et al. (1993) |
| JB4.5 (NT-COI\_240\_R) | TAAAGAAAGAACATAATGAAAATG |  |  |  |
| NemCOIintF | TGATTGGTGGTTTTGGTAA | COI | ~650 | Casiraghi et al. (2001) |
| NemCOIintR | ATAAGTACGAGTATCAATATC |  |  |  |

**Supplementary Table 2: Composition of PCR reaction and cycler conditions for each primer pair.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Primer name** | **Nem\_18S** | | | **Mm527\_1514** | | | **Nem\_ITS+** | | | **NT\_COI** | | | **NemCOIint** | | |
| Mastermix conditions [20µl] | Final concentration ; Volume [µl] | | | | | | | | | | | | | | |
| dH2O | - | 13.3 | | - | 12.9 | | - | 12.9 | | - | 11.7 | | - | 12.9 | |
| 10x DreamTaq Buffer | - | 2 | | - | 2 | | - | 2 | | - | 2 | | - | 2 | |
| dNTPS | 0.8 mM | 1.6 | | 0.5 mM | 1 | | 0.5 mM | 1 | | 0.5 mM | 1 | | 0.5 mM | 1 | |
| Forward primer | 0.5 µM | 1 | | 0.5 µM | 1 | | 0.5 µM | 1 | | 0.5 µM | 1 | | 0.5 µM | 1 | |
| Reverse Primer | 0.5 µM | 1 | | 0.5 µM | 1 | | 0.5 µM | 1 | | 0.5 µM | 1 | | 0.5 µM | 1 | |
| MgCl2 | - | 0 | | - | 0 | | - | 0 | | 1.5 mM | 1.2 | | - | 0 | |
| DreamTaq DNA Polymerase | 0.025 U/µl | 0.1 | | 0.025 U/µl | 0.1 | | 0.025 U/µl | 0.1 | | 0.025 U/µl | 0.1 | | 0.025 U/µl | 0.1 | |
| Template DNA | - | 1 | | - | 2 | | - | 2 | | - | 2 | | - | 2 | |
| Cycler conditions | Time [min]; Temperature [C°]; Cycles | | | | | | | | | | | | | | |
| Initial activation step | 05:00 | 94 |  | 05:00 | 95 |  | 01:00 | 94 |  | 10:00 | 95 |  | 03:00 | 95 |  |
| Denaturation step | 00:30 | 94 | 35 | 00:30 | 94 | 35 | 00:30 | 94 | 30 | 00:30 | 94 | 35 | 00:45 | 94 | 40 |
| Annealing | 00:30 | 54 |  | 00:30 | 51 |  | 00:30 | 55 |  | 00:30 | 50 |  | 00:45 | 52 |  |
| Extension | 01:00 | 72 |  | 01:00 | 72 |  | 00:30 | 72 |  | 00:30 | 72 |  | 01:30 | 72 |  |
| Final extension step | 10:00 | 72 |  | 10:00 | 72 |  | 07:00 | 72 |  | 10:00 | 72 |  | 10:00 | 72 |  |

**Supplementary Table 3: Nematode reference sequences from different gene regions available in NCBI used in phylogenetic analyses.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Host species** | **Location** | **Gene region** | **Lenght (bp)** | **GenBank accession no.** | **Reference** |
| *Dirofilaria immitis* | *Culicidae sp.* | USA | 18S | 1329 | AF182647 | Watts et al. (1999) |
| *Dirofilaria repens dog* | *Canis familiaris* | India | 18S | 823 | MG780293 | Pradeep et al. (2018) |
| *Dirofilaria repens HS* | *Homo sapiens* | Japan | 18S | 1744 | AB973229 | Suzuki et al. (2015) |
| *Gongylonema neoplasticum* | *Rattus norvegicus* | Thailand | 18S | 1756 | LC331000 | Setsuda et al. (2018) |
| *Gongylonema pulchrum* | *Saimiri boliviensis* | Japan | 18S | 1756 | AB495401 | Halajian et al. (2010) |
| *Mastophorus muris rat* | *Rattus norvegicus* | South Africa | 18S | 1134 | MG356473 | Julius, et al. (2018) |
| *Mastophorus muris wildcat* | *Felis silvestris silvestris* | Germany | 18S | 1756 | MG818763 | Present study |
| *Mastophorus muris BER008* | *Apodemus flavicollis* | Germany | 18S | 779 | MN08629 | Present study |
| *Protospirura sp.* | *Mastomys coucha* | South Africa | 18S | 1677 | KY462830 | Julius, et al. (2018) |
| *Gongylonema sp. FE2012* | *Otus scops* | Spain | 18S | 699 | JX401925 | Esperon et al. (2013) |
|  |  |  |  |  |  |  |
| *Dirofilaria immitis* | *Canis familiaris* | Korea | COI | 441 | AF181893 | Lee et al. unpublished |
| *Dirofilaria immitis* | *Canis familiaris* | Australia | COI | 858 | AJ537512 | Hu et al. (2003) |
| *Dirofilaria repens HS* | *Homo sapiens* | Japan | COI | 715 | AB973225 | Suzuki et al. (2015) |
| *Gonyglynema neoplasticum* | *Rattus norvegicus* | Thailand | COI | 818 | LC331040 | Setsuda et al. (2018) |
| *Gongylonema pulchrum* | *Saimiri sciureus* | Japan | COI | 369 | AB513730 | Makouloutou et al. (2013) |
| *Mastophorus muris rat* | *Rattus norvegicus* | South Africa | COI | 398 | MG386206 | Julius, et al. (2018) |
| *Mastophorus muris BER008* | *Apodemus flavicollis* | Germany | COI | 804 | MK867480 | Present study |
| *Mastophorus muris wildcat* | *Felis silvestris silvestris* | Germany | COI | 858 | MG821081 | Present study |
| *Mastophorus muris Sigmodon* | *Sigmodon hispidus* | USA | COI | 710 / 710 / 655 | MH782850 / MH782851 /MH782852 | Thompson et al. (2019) |
| *Protospirura muricola* | *Gorilla sp.* | Central African Republic | COI | 591 | KP760207 | Lefoulon et al. (2015) |
| *Protospirura muricola* | *Gorilla gorilla* | Cameroon | COI | 474 | MN890122/ MN890123/ MN890124 | Gaillard et al., (2020) |
| *Protospirura sp.* | *Cercopithecus nictitans* | Cameroon | COI | 474 | MN890114/ MN890093/ MN890094 | Gaillard et al., (2020) |
|  |  |  |  |  |  |  |
| *Dirofilaria immitis* | *Canis familiaris* | Taiwan | ITS | 1132 | AF217800 | Mar et al. (2002) |
| *Dirofilaria immitis solgi* | *Canis familiaris* | Iran | ITS | 1132 | KY863453 | Solgi et al. (2018) |
| *Dirofilaria repens HS* | *Homo sapiens* | Japan | ITS | 1351 | AB973229 | Suzuki et al. (2015) |
| *Dirofilaria repens DR2* | NA | Thailand | ITS | 560 | AY621480 | Nuchprayoon et al. unpublished |
| *Gonyglynema neoplasticum* | *Rattus norvegicus* | Thailand | ITS | 1351 | LC331000 | Setsuda et al. (2018) |
| *Gongylonema pulchrum* | *Saimiri boliviensis* | Japan | ITS | 1351 | AB495401 | Halajian et al. (2010) |
| *Mastophorus muris wildcat* | *Felis silvestris silvestris* | Germany | ITS | 1351 | MG818763 | Present study |
| *Protospirura sp. S19* | *Rattus rattus* | Spain | ITS | 892 | JF514771 | Feliu, C. unpublished |
| *Protospirura sp. S17* | *Rattus rattus* | Spain | ITS | 888 | JF514769 | Feliu, C. unpublished |
| *Protospirura sp. S45* | *Rattus rattus* | Spain | ITS | 892 | JF514770 | Feliu, C. unpublished |
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**Supplementary Table 4: Dentition pattern per sample observed by SEM.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample ID** | **Sex** | **Trilobed pseudolabia** | | | | | | | | | | | | | | |
|  |  | **left** | | | | | **middle** | | | | | **right** | | | | |
| AA\_0256f\_7 | F | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv |
|  |  | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv |
| AA\_0256m\_8 | M | nv | 2 | 1 | nv | nv | 1 | 5 | 1 | 4 | 1 | nv | 2 | 1 | 3 | 1 |
|  |  | nv | 2 | 1 | nv | nv | 1 | 4 | 1 | 3 | 1 | nv | nv | 1 | 2 | 1 |
| AA\_0348f\_9 | F | nv | 3 | nv | nv | nv | 1 | 3 | 1 | 4 | 1 | nv | nv | nv | nv | nv |
|  |  | nv | nv | 1 | nv | nv | 1 | 2 | 1 | 3 | 1 | nv | nv | 1 | 3 | nv |
| AA\_0348m\_10 | M | nv | 2 | 1 | 2 | 1 | nv | 2(+) | 1 | 5 | 1 | nv | nv | 1 | nv | nv |
|  |  | nv | nv | nv | nv | nv | 1 | 4 | 1 | 2(+) | 1 | nv | nv | 1 | nv | nv |
| AA\_0351f\_11 | F | nv | 2 | 1 | nv | nv | 1 | 3 | 1 | 3 | 1 | 1 | nv | 1 | 2 | 1 |
|  |  | nv | 2 | 1 | 3 | nv | 1 | 4 | 1 | 4 | 1 | 1 | 3 | 1 | 2 | 1 |
| AA\_0351m\_12 | M | 1 | 2 | 1 | nv | nv | 1 | nv | 1 | nv | 1 | nv | nv | 1 | nv | nv |
|  |  | nv | nv | 1 | 3 | nv | 1 | nv | 1 | nv | 1 | nv | nv | nv | 2 | nv |
| AA\_0349f\_6 | F | nv | nv | nv | nv | nv | 1 | 5 | 1 | nv | | | | 1 | 2(+) | nv |
|  |  | nv | nv | 1 | nv | nv | 1 | 3 | 1 | 2 | 1 | 1 | nv | 1 | 3 | nv |
| AA\_0349m\_7 | M | nv | nv | 1 | 1(+) | nv | 1 | 3 | 1 | 2 | 1 | nv | nv | 1 | 2 | 1 |
|  |  | nv | nv | 1 | nv | 1 | 1 | 4 | 1 | 3 | 1 | nv | 3 | 1 | 2 | nv |
| AA\_0506f\_8 | F | nv | 1(+) | nv | 2(+) | 1 | 1 | 5 | 1 | 4 | 1 | nv | nv | 1 | 2 | nv |
|  |  | 1 | 2 | 1 | nv | nv | nv | nv | 1 | 4 | 1 | 1 | 2 | 1 | 2 | 1 |
| AA\_0348A | F | 1 | 3 | 1 | 3 | nv | 1 | 3 | 1 | 5 | 1 | nv | nv | 1 | 1(+) | nv |
|  |  | nv | 2(+) | 1 | nv | nv | nv | 2(+) | 1 | 2(+) | nv | nv | nv | nv | 2 | 1 |
| AA\_0348C | F | 1 | nv | 1 | nv | nv | nv | nv | nv | nv | 1 | nv | nv | 1 | nv | nv |
|  |  | nv | nv | 1 | nv | nv | 1 | 4 | 1 | 2(+) | nv | nv | nv | nv | nv | nv |
| AA\_0256D | F | 1 | 2 | 1 | nv | nv | nv | 4 | 1 | 2(+) | nv | nv | nv | 1 | 2 | 1 |
|  |  | nv | 2 | 1 | 2 | nv | 1 | 5 | 1 | 3 | nv | 1 | 2 | 1 | 3 | nv |
|  |  | 1 | (2-3) | 1 | (2-3) | 1 | 1 | (2-5) | 1 | (2-5) | 1 | 1 | (2-3) | 1 | (2-3) | 1 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Mm\_005\_MG | F | nv | 1(+) | 1 | nv | nv | nv | 7 (3-1-3) | | | nv | nv | 1(+) | 1 | 2 | nv |
|  |  | nv | 2 | 1 | nv | nv | nv | 7(3-1-3) | | | nv | nv | nv | 1 | nv | nv |
| Mm\_005\_MG | M | nv | 2 | 1 | 3 | nv | nv | 9 (4-1-4) | | | nv | nv | 3 | 1 | 2 | nv |
|  |  | nv | 2(+) | 1 | 3 | nv | nv | 7 (2-1-4) | | | nv | nv | nv | 1 | 2 | nv |
|  |  |  | (2-3) | 1 | (2-3) |  |  | (2-4) | 1 | (2-4) |  |  | (2-3) | 1 | (2-3) |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Mm\_008\_AF | F | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv |
|  |  | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv | nv |
| Mm\_008\_AF | M | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
|  |  | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
|  |  | NA | | | | | | | | | | | | | | |

Each row represents results for one specimen with two trilobed pseudolabia. Host origin of specimens is specified as follows: AA – *Mus* , AF – *Apodemus* , MG- *Myodes.* F- Female, M- Male, nv - not visible, NA- not available.