|  |
| --- |
|  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

|  |  |  |
| --- | --- | --- |
|  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |

|  |  |  |
| --- | --- | --- |
|  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

**Table S1.** PCR mix, primers and conditions used for each molecular marker sequenced in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **ITS1** | **ITS2** | ***Cyt*b** | ***cox*1** |
|  |  | **PCR Mix** |  |  |
| Forward Primer (10 μM)  | 5 µl | 5 µl | 5 µl | 5 µl |
| Reverse Primer (10 μM)  | 5 µl | 5 µl | 5 µl | 5 µl |
|  Template DNA  | 5 µl | 5 µl | 5 µl | 5 µl |
| *goTaq* G2 Green Master Mix DNA polymerase |  25 µl | 25 µl | 25 µl | 25 µl |
| Autoclaved distilled water to | 50 µl | 50 µl | 50 µl | 50 µl |
|  |  | **PCR Primers** |  |  |
| Forward Primer | NC5 (Gasser *et al*., 1996) | senITS2 (Vobis *et al*., 2004) | CytbF (Dittmar & Whiting, 2003) | Kmt6 (Zhu *et al*., 2015) |
| Reverse Primer | ITS1rev (Marrugal *et al*., 2013) | ITS2R (Luchetti *et al*., 2007)  | A5F (Dittmar & Whiting, 2003) | HCO2198 (Folmer *et al*., 1994) |
|  |  | **PCR Conditions** |  |  |
|  Initial Denaturing | 94º C for 5´ | 94º C for 5´ | 95º C for 12´ | 96º C for 2´ |
| Number of cycles | 35  | 35  | 30 | 40 |
| Denaturing | 94º C for 30´´ | 94º C for 60´´ | 95º C for 30´´ | 94º C for 30´´ |
| Annealing | 58º C for 30´´ | 55º C for 60´´ | 40º C for 30´´ | 50º C for 30´´ |
| Primer extension | 72º C for 90´´ | 72º C for 60´´ | 68º C for 2´ | 72º C for 60´´ |
| Final extension | 72º C for 5´ | 72º C for 10´ | 68º C for 7´ | 72º C for 7´ |

**Table S2.** List of taxa used in the analysis, including GenBank accession numbers and host information.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Species** | **Family** | **Host** | **Accession number** | **Gen Region** | **Sequence****length** |
| *Ophthalmopsylla kiritschenkoi* | Leptopsyllidae | Unknown | GQ161960 | ITS2 | 474 |
| *Ophthalmopsylla extrema* | Leptopsyllidae | Unknown | GQ161956 | ITS2 | 466 |
| *Amphipsylla quadratoides quadratoides* | Leptopsyllidae | Unknown | AY072642 | ITS2 | 497 |
| *Leptopsylla* sp. | Leptopsyllidae | Unknown | EF504221 | ITS2 | 459 |
| *Leptopsylla* sp. | Leptopsyllidae | Unknown | EF504223 | ITS2 | 449 |
| *Neopsylla siboi* | Ctenophthalmidae | Unknown | AF353113 | ITS2 | 479 |
| *Neopsylla teratura* | Ctenophthalmidae | Unknown | AF353122 | ITS2 | 479 |
| *Neopsylla stevensi* | Ctenophthalmidae | Unknown | AY337033 | ITS2 | 479 |
| *Neopsylla specialis* | Ctenophthalmidae | Unknown | AF353120 | ITS2 | 479 |
| *Xenopsylla cheopis* | Pulicidae | *Rattus* sp. | DQ295061 | ITS2 | 356 |
| *Xenopsylla cheopis* | Pulicidae | *Rattus* sp. | DQ295059 | ITS2 | 356 |
| *Xenopsylla cheopis* | Pulicidae | *Rattus* sp. | LT604121 | ITS2 | 358 |
| *Ctenocephalides felis* | Pulicidae | *Canis lupus familiaris* | LN827903 | ITS2 | 327 |
| *Ctenocephalides felis* | Pulicidae | *Canis lupus familiaris* | LT853876 | ITS2 | 327 |
| *Ctenocephalides canis* | Pulicidae | *Canis lupus familiaris* | LN827905 | ITS2 | 327 |
| *Ctenocephalides canis* | Pulicidae | *Canis lupus familiaris* | LN864485 | ITS2 | 327 |
| *Archaeopsylla erinacei* | Pulicidae | *Erinaceus europaeus* | LT703438 | ITS2 | 360 |
| *Archaeopsylla erinacei* | Pulicidae | *Erinaceus europaeus* | LT604114 | ITS2 | 361 |
| *Pulex irritans* | Pulicidae | *Lycalopex culpaeus* | LT797451 | ITS2 | 324 |
| *Pulex irritans* | Pulicidae | - | LT797448 | ITS2 | 322 |
| *Pulex irritans* | Pulicidae | - | LT797449 | ITS2 | 322 |
| *Tunga penetrans* | Tungidae | *Homo sapiens* | DQ844716 | ITS2 | 471 |
| *Tunga penetrans* | Tungidae | *Homo sapiens* | DQ844724 | ITS2 | 473 |
| *Tunga trimamillata* | Tungidae | Unknown | AY425820 | ITS2 | 470 |
| *Stenoponia tripectinata tripectinata* | Stenoponiidae | *Mus musculus* | LK937042 | ITS2 | 332 |
| *Stenoponia tripectinata tripectinata* | Stenoponiidae | *Mus musculus* | LK937039 | ITS2 | 332 |
| *Stenoponia tripectinata tripectinata* | Stenoponiidae | *Mus musculus* | LK937038 | ITS2 | 332 |
| *Citellophilus tesquorum dzetysuensis* | Ceratophyllidae | Unknown | EU770316 | ITS2 | 332 |
| *Citellophilus tesquorum altaicus* | Ceratophyllidae | Unknown | EU770312 | ITS2 | 332 |
| *Nospsyllus fasciatus* | Ceratophyllidae | *Apodemus sylvaticus* | LT158059 | ITS2 | 318 |
| *Nosopsyllus fasciatus* | Ceratophyllidae | Muridae | LT158060 | ITS2 | 318 |
| *Nosopsyllus barbarus* | Ceratophyllidae | *Rattus* sp. | LN881537 | ITS2 | 318 |
| *Panorpa meridionalis* | Panorpidae | - | LT604124 | ITS2 | 1.121 |
| *Echidnophaga gallinacea* | Pulicidae | *Oryctolagus cuniculus* | JN008921 | *Cox*1 | 650 |
| *Echidnophaga myrmecobii* | Pulicidae | *Oryctolagus cuniculus* | JN008919 | *Cox*1 | 649 |
| *Echidnophaga iberica* | Pulicidae | *Oryctolagus cuniculus* | KF479239 | *Cox*1 | 658 |
| *Echidnophaga* sp. | Pulicidae | Mammal | JN008922 | *Cox*1 | 654 |
| *Xenopsylla cunicularis* | Pulicidae | *Oryctolagus cuniculus* | KF479238 | *Cox*1 | 658 |
| *Pulex irritans* | Pulicidae | *Meles meles* | KF479246 | *Cox*1 | 658 |
| *Pulex irritans* | Pulicidae | *Homo sapiens* | KF479247 | *Cox*1 | 658 |
| *Pulex* *irritans* | Pulicidae | *Canis lupus familiaris* | KY048351 | *Cox*1 | 658 |
| *Pulex irritans* | Pulicidae | Jackal | MG668627 | *Cox*1 | 489 |
| *Pulex irritans* | Pulicidae | Fox | MG668624 | *Cox*1 | 489 |
| *Pulex irritans* | Pulicidae | - | LT797468 | *Cox*1 | 658 |
| *Pulex irritans* | Pulicidae | - | LT797469 | *Cox*1 | 658 |
| *Pulex irritans* | Pulicidae | *Lycalopex griseus* | LT797466 | *Cox*1 | 658 |
| *Pulex irritans* | Pulicidae | *Lycalopex culpaeus* | LT797467 | *Cox*1 | 658 |
| *Spilopsyllus cuniculi* | Pulicidae | *Oryctolagus cuniculus* | KF479236 | *Cox*1 | 658 |
| *Spilopsyllus cuniculi* | Pulicidae | *Oryctolagus cuniculus* | KF479237 | *Cox*1 | 658 |
| *Archaeopsylla erinacei* | Pulicidae | *Erinaceus europaeus* | LT604116 | *Cox*1 | 658 |
| *Archaeopsylla erinacei* | Pulicidae | *Erinaceus europaeus* | LT604115 | *Cox*1 | 658 |
| *Archaeopsylla erinacei* | Pulicidae | *Erinaceus europaeus* | LT627349 | *Cox*1 | 658 |
| *Archaeopsylla erinacei* | Pulicidae | *Erinaceus europaeus* | LT703440 | *Cox*1 | 658 |
| *Ctenocephalides felis* | Pulicidae | *Canis lupus familiaris* | LN827896 | *Cox*1 | 600 |
| *Ctenocephalides felis felis* | Pulicidae | *Felis catus* | KF684891 | *Cox*1 | 601 |
| *Ctenocephalides felis strongylus* | Pulicidae | *Canis lupus familiaris* | KF684876 | *Cox*1 | 601 |
| *Ctenocephalides orientis* | Pulicidae | *Canis lupus familiaris* | KF684871 | *Cox*1 | 601 |
| *Ctenocephalides canis* | Pulicidae | *Canis lupus familiaris* | KP684210 | *Cox*1 | 658 |
| *Ctenocephalides canis* | Pulicidae | *Canis lupus familiaris* | LN827901 | *Cox*1 | 600 |
| *Stenoponia tripectinata tripectinata* | Stenoponiidae | *Mus musculus* | LK937072 | *Cox*1 | 677 |
| *Stenoponia tripectinata tripectinata* | Stenoponiidae | *Mus musculus* | LK937071 | *Cox*1 | 677 |
| *Stenoponia tripectinata tripectinata* | Stenoponiidae | *Mus musculus* | LK937073 | *Cox*1 | 677 |
| *Nosopsyllus fasciatus* | Ceratophyllidae | *Crocidura russula* | LT158040 | *Cox*1 | 658 |
| *Nosopsyllus fasciatus* | Ceratophyllidae | *Apodemus sylvaticus* | LT158041 | *Cox*1 | 658 |
| *Nosopsyllus barbarus* | Ceratophyllidae | *Rattus* sp. | LN881549 | *Cox*1 | 658 |
| *Nosopsyllus barbarus* | Ceratophyllidae | *Rattus* sp. | LN881550 | *Cox*1 | 658 |
| *Coptopsylla lamellifer* | Coptopsyllidae | Rodent | MG138322 | *Cox*1 | 658 |
| *Coptopsylla lamellifer* | Coptopsyllidae | Rodent | MG138320 | *Cox*1 | 658 |
| *Neotyphloceras crassispina* | Ctenophthalmidae | *Abrocoma bennetti* | KM890944 | *Cox*1 | 1,197 |
| *Chiliopsylla allophyla* | Ctenophthalmidae | Unknown | KM891001 | *Cox*1 | 1,244 |
| *Corrodopsylla curvata curvata* | Ctenophthalmidae | Unknown | KR142880 | *Cox*1 | 638 |
| *Corrodopsylla curvata curvata* | Ctenophthalmidae | Unknown | KM529504 | *Cox*1 | 615 |
| *Ctenophthalmus* sp. | Ctenophthalmidae | Unknown | KM891003 | *Cox*1 | 630 |
| *Ctenophthalmus calceatus cabirus* | Ctenophthalmidae | *Lemmniscomys striatus* | MH142441 | *Cox*1 | 659 |
| *Ctenophthalmus dolichus dolichus* | Ctenophthalmidae | *Rattus* sp. | MF000657 | *Cox*1 | 657 |
| *Ctenophthalmus congeneroides congeneroides* | Ctenophthalmidae | Unknown | KM890918 | *Cox*1 | 1,182 |
| *Ctenophthalmus cryptotis* | Ctenophthalmidae | Unknown | KM890939 | *Cox*1 | 1,218 |
| *Panorpa meridionalis* | Panorpidae | - | LT604125 | *Cox*1 | 658 |
| *Panorpa meridionalis* | Panorpidae | *-* | LT604126 | *Cox*1 | 658 |
| *Stenoponia tripectinata tripectinata* | Stenoponiidae | *Mus musculus* | LN897473 | *Cyt*b | 374 |
| *Ctenocephalides felis* | Pulicidae | *Canis lupus familiaris* | LN897470 | *Cyt*b | 374 |
| *Ctenocephalides felis felis* | Pulicidae | Unknown | KM890759 | *Cyt*b | 369 |
| *Ctenocephalides canis* | Pulicidae | *Canis lupus familiaris* | LN897471 | *Cyt*b | 374 |
| *Ctenocephalides felis* | Pulicidae | *Canis lupus familiaris* | LT853878 | *Cyt*b | 374 |
| *Xenopsylla cheopis* | Pulicidae | *Rattus* sp. | LT604122 | *Cyt*b | 374 |
| *Archaeopsylla erinacei erinacei* | Pulicidae | Unknown | KM890725 | *Cyt*b | 369 |
| *Archaeopsylla erinacei* | Pulicidae | *Erinaceus europaeus* | LT604120 | *Cyt*b | 374 |
| *Archaeopsylla erinacei* | Pulicidae | *Erinaceus europaeus* | LT604117 | *Cyt*b | 374 |
| *Archaeopsylla erinacei* | Pulicidae | *Erinaceus europaeus* | LT627350 | *Cyt*b | 374 |
| *Pulex irritans* | Pulicidae | *Lycalopex culpaeus* | LT797476 | *Cyt*b | 374 |
| *Pulex irritans* | Pulicidae | *Lycalopex griseus* | LT797480 | *Cyt*b | 374 |
| *Pulex irritans* | Pulicidae | - | LT797473 | *Cyt*b | 374 |
| *Pulex irritans* | Pulicidae | - | LT797474 | *Cyt*b | 374 |
| *Nosopsyllus barbarus* | Ceratophyllidae | *Rattus* sp | LN897460 | *Cyt*b | 374 |
| *Nosopsyllus barbarus* | Ceratophyllidae | *Rattus* sp | LN897462 | *Cyt*b | 374 |
| *Nosopsyllus fasciatus* | Ceratophyllidae | Muridae | LT158049 | *Cyt*b | 374 |
| *Nosopsyllus fasciatus* | Ceratophyllidae | *Apodemus sylvaticus* | LT158043 | *Cyt*b | 374 |
| *Nosopsyllus iranis theodori* | Ceratophyllidae | *Gerbillus dasyurus* | KM890603 | *Cyt*b | 369 |
| *Nosopsyllus laeviceps ellobii* | Ceratophyllidae | Unknown | KM890720 | *Cyt*b | 369 |
| *Stephanocircus dasyuri* | Stephanocircidae | Unknown | KM890619 | *Cyt*b | 369 |
| *Stephanocircus pectinipes* | Stephanocircidae | Unknown | KM890658 | *Cyt*b | 369 |
| *Pygiopsylla hoplia* | Pygiopsyllidae | Unknown | KM890657 | *Cyt*b | 369 |
| *Metastivalius mordax* | Stivaliidae | Unknown | KM890628 | *Cyt*b | 369 |
| *Parastivalius novaeguinae* | Stivaliidae | Unknown | KM890629 | *Cyt*b | 369 |
| *Neotyphloceras crassispina chilensis* | Ctenophthalmidae | Unknown | KM890613 | *Cyt*b | 369 |
| *Neotyphloceras crassispina* | Ctenophthalmidae | Unknown | KM890677 | *Cyt*b | 369 |
| *Chiliopsylla allophyla* | Ctenophthalmidae | Unknown | KM890736 | *Cyt*b | 369 |
| *Ctenophthalmus congeneroides* | Ctenophthalmidae | Unknown | KM890651 | *Cyt*b | 369 |
| *Ctenophthalmus cryptotis* | Ctenophthalmidae | Unknown | KM890672 | *Cyt*b | 369 |
| *Ctenophthalmus sanborni* | Ctenophthalmidae | Unknown | KM890607 | *Cyt*b | 330 |
| *Ctenophthalmus* sp. | Ctenophthalmidae | Unknown | KM890749 | *Cyt*b | 369 |
| *Panorpa meridionalis* | Panorpidae | - | LT604127 | *Cyt*b | 374 |
| *Panorpa meridionalis* | Panorpidae | - | LT604128 | *Cyt*b | 374 |