

“Multiple lineages of lice pass through the K-Pg boundary”

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Electronic Supplementary Material

This supplement provides an expanded description of the analytical methods used in this study. We also present additional supplementary figures showing the non-rate smoothed phylogeny (including posterior probabilities) and a figure based on an alternative dating method in support of our central conclusion. A table containing the host associations and GenBank accession numbers of the sampled taxa is also included.

Supplementary Methods

Sequencing

DNA was isolated from louse specimens using the DNAeasy Tissue Kit (QIAGEN Inc., Valencia, California) using louse specific protocols [1]. After DNA extraction, lice were mounted on slides and retained as vouchers. Due to the large number of taxa obtained from previous studies, we focused our laboratory work on three genes that are well represented in GenBank. Portions of the nuclear 18S rRNA (18S; 1536 base pairs [bp]) and elongation factor 1 alpha (EF-1 α ; 429 bp) genes and the mitochondrial cytochrome *c* oxidase subunit 1 (COI; 381 bp) gene were amplified and sequenced using primers 18Sai and 18Sbi [2], EF1For3 and Cho10 [3], and L6625 and H7005 [4], respectively. Double-stranded PCR amplifications, PCR purification, and sequencing of these genes were undertaken following protocols detailed in Light and Reed [5]. Sequences were edited

using Sequencher v. 4.2.2 (Gene Codes Corporation, Ann Arbor, Michigan) and primer sequences were removed. Sequences were trimmed in reference to the translated protein sequence using Se-AL v2.01a11 [6] and MacClade 4.06 [7]. The protein coding genes EF-1 α and COI were aligned by eye using Se-AL v2.0a11 and louse 18S rRNA sequences were aligned using CLUSTAL W [8] and rRNA secondary structure, using the procedure outlined in Johnson et al [9]. Longer sequences obtained from GenBank were pruned for maximum overlap with sequences generated herein. All sequences are available in GenBank (Table S3) and alignments are available on TreeBase (<http://purl.org/phylo/treebase/phylows/study/TB2:S11088>; Submission ID 11088).

Phylogenetic Analysis

Bayesian phylogenetic analyses were performed in MrBayes 3.12 [10]. Model parameters were treated as unknown variables with uniform priors and were estimated as part of the analysis. Bayesian analyses were initiated from random starting trees, run for 10 million generations with 4 incrementally heated chains, and sampled at intervals of 1000 generations. Two independent Bayesian analyses were run to avoid entrapment on local optima, and log-likelihood scores were compared for convergence so that burn-in generations (the first 3000 trees) could be discarded. Tracer v1.4 [11] was used to evaluate stability of all parameter estimates following removal of burn-in generations. The phylogeny (and posterior probabilities) resulting from the Bayesian analysis is shown in Figure S1.

Estimates of Divergence Times

Divergence times were estimated using Bayesian approaches implemented in MultiDivTime [12, 13] and BEAST v1.5.3 [14, 15], which assume that rates of evolution are autocorrelated and uncorrelated, respectively. In MultiDivTime, model parameters for the F84+ Γ model were estimated for each gene individually using the *baseml* program in PAML v3.14 [16]. These parameters were used in the program *estbranches* [12, 13] to estimate the ML and variance-covariance matrix (also using the F84+ Γ model) of the branch length estimates. Lastly, the program MultiDivTime [12, 13], utilizing the output files from *estbranches* and implementing Markov chain Monte Carlo sampling, was used to estimate prior and posterior distribution of both the substitution rates and the estimated divergence time for each node. The mean and standard deviation for the prior distribution of the rate of evolution at the ingroup node (*rtrate* and *rtratesd*) was determined following the procedure of Jansa et al. (2006). The node constraints described below were applied on the appropriate tree node as minimum and maximum bounds. The Markov chain was initialized by randomly selecting the initial parameter value and each chain was sampled every 100 cycles for 10^6 generations with a burn-in of 10^5 cycles. Analyses were performed twice to ensure stationarity.

In contrast to MultiDivTime, BEAST uses a Bayesian relaxed molecular clock while incorporating tree uncertainty in the MCMC process to infer divergence times. In BEAST, data were analyzed in a partitioned framework (by gene and codon position as in Light et al. [17]) and analyses were initialized with the chronogram resulting from the MultiDivTime analyses. Node constraints were assigned uniform prior distributions as described below. A Yule process speciation prior and an uncorrelated lognormal model

of rate variation were implemented in the analysis [14]. Posterior probability distributions of node ages were obtained for the multigene alignment with analyses performed in a concatenated and partitioned framework (all model parameters were unlinked across partitions). Best-fit models of nucleotide substitution for each data set were the same as those identified above as part of the phylogenetic analyses using MrModelTest [18]. Two separate MCMC analyses were run for 30,000,000 generations (burnin 10%) with parameters sampled every 1000 steps. Independent runs were combined using LogCombiner v.1.5.3 [15]. Tracer v1.5 [11] was used to measure the effective sample size of each parameter (all resulting effective sample sizes exceeded 100) and calculate the mean and upper and lower bounds of the 95% highest posterior density interval (95% HPD) for divergence times. Tree topologies were assessed using TreeAnnotator v.1.5.3 [15] and FigTree v.1.3.1 [19].

Calibration Points

Sampled taxa span eight calibrations points (Figure 1 & S2), two of which are based on insect fossils and the remainder derived from host fossil record. The latter correspond to cospeciation points identified in independent host-louse cophylogenetic studies. These points mark a common temporal event (the co-divergence of a host and parasite lineage) and allow us to calibrate fixed points in both the host and parasite phylogeny [20] as exemplified by a recent study on blood sucking lice [17].

A. Fossil *Megamenopon* louse, 44 Myr (minimum): Calibration of this node is based on the minimum age of the only parasitic fossil louse [21]. This taxon is phylogenetically

placed within the *Austromenopon*-complex of the louse suborder Amblycera [22] and is the corresponding node is assigned a minimum age of 44 Myr.

B. Fossil Liposcelid book-louse, 100 Myr (minimum): Based on the mid- Cretaceous finds of fossilised book-lice (“Psocoptera”: Liposcelididae) [23], which are candidate sister taxa to the Amblycera [24].

C. Range between the oldest pocket gopher fossil and its closest sister taxon, 4.75 - 30 Myr: Pocket gophers (Geomyidae) and their chewing lice are widely recognised as the model system for host-parasite cospeciation [4, 25]. Based on fossil data the host clade are estimated to have split from their closest extant relatives 4.75 - 30 Myr [26].

D. Range between the oldest fossil Diomedeidae and the oldest fossil procellariform, 37 - 53.25 Myr: Albatrosses are host to an in situ radiation of several chewing louse clades, each of which showing a high degree of cospeciation with there hosts [27]. This is particularly evident in the genus louse *Paraclisis*, which shows a long and shared coevolutionary history with its host. We calibrate the divergence between *Paraclisis diomedea* and *P. hyalina* based on split age range of the oldest fossil Diomedeidae and the oldest fossil procellariform [28].

E. Oldest columbid fossil, 19.8 Myr (minimum): This is based on the minimum age of the oldest Columbiform fossil [28]. The split between sampled louse species *Columbicola columbae* and *Columbicola passerinae* spans the base of the phylogeny of *Columbicola*

and corresponds with the basal split with their columbid hosts [29]. This clade has undergone extensive cospeciation with their hosts and the corresponding basal split is a cospeciation event [30].

F. Oldest Pelecaniform fossil, 49 Myr (minimum): Pelecaniform birds have undergone extensive cospeciation with the louse genus *Pectinopygus* [31]. The sampled *Pectinopygus* species span close to the base phylogeny of pelecaniform birds, and this is a cospeciation event [31]. Accordingly this node in the louse phylogeny is assigned a minimum ago of 49 Myr based on the fossil sulid specimen *Masillastega rectirostris* described by Mayr [32].

G. Old World monkeys and apes, 20 - 25 Myr: Based on fossil and molecular data as established by Reed et al [33] and Light et al [17], this corresponds to the split between Old World monkeys and apes. A calibration of 20-25 Ma was used to represent the split between Old World monkey lice (*Pedicinus* spp.) and hominoid-specific lice (*Pthirus* and *Pediculus*).

H. Chimpanzees and humans, 5 - 7 Myr: A calibration of 5-7 Ma corresponding to the split between chimpanzees and humans was used for the split between the head and body lice of humans (*Pediculus humanus* ssp.) and the chimpanzee louse (*Pediculus schaeffi*). The fossil and molecular data used to justify this calibration are detailed in Reed et al. [33] and Light & Reed [5].

Supplementary References

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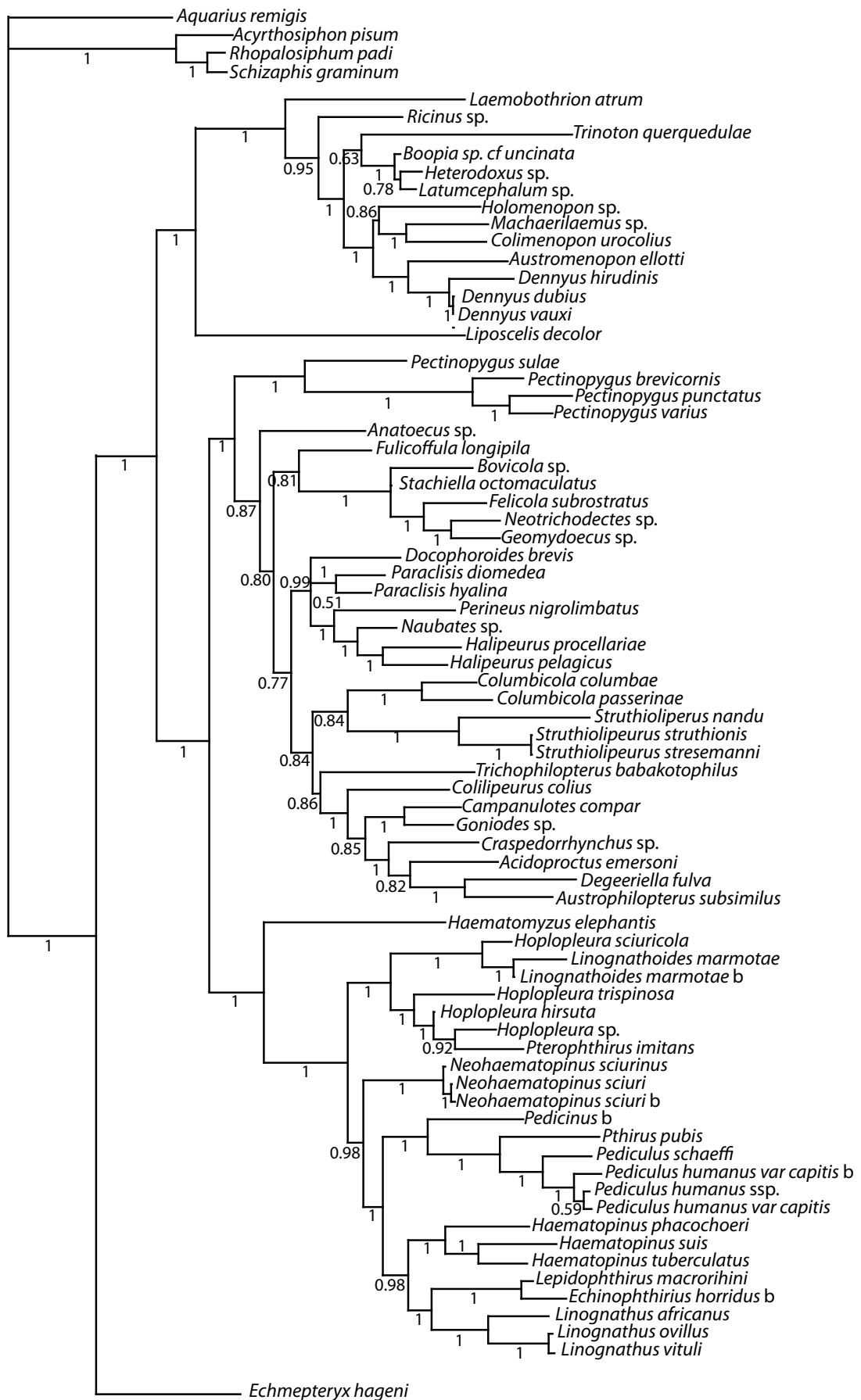
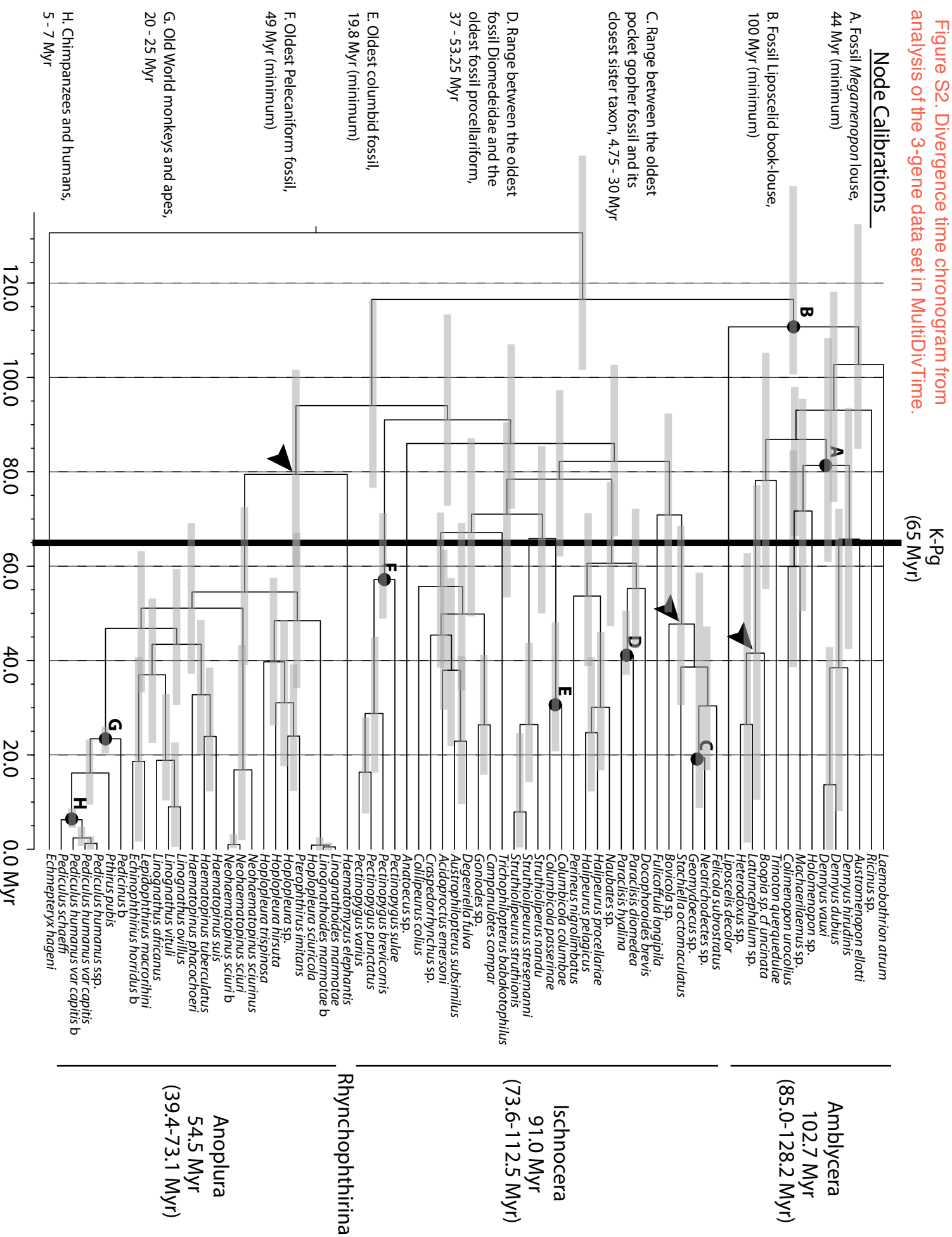


Figure S1. Bayesian phylogram showing all posterior probability values.

— 0.01 substitutions/site

Figure S2. Divergence time chronogram from analysis of the 3-gene data set in MultiDivTime.



GenBank accession numbers

Taxa used in this study with host associations and corresponding GenBank accession numbers. An 'X' denotes missing data. Asterisks beside identifiers indicate 18S sequences generated from a study by Barker *et al.* [34].

Louse Species	Host Species	18S	EF1a	COI
Outgroup				
<i>Acyrtosiphon pisum</i>	NA	X62623	AF219737	AF077776
<i>Aquarius remigis</i>	NA	U15691	X	AF200251
<i>Rhopalosiphum padi</i>	NA	AF487719	AY219719	X
<i>Schizaphis graminum</i>	NA	U27827	AF068479	AF220522
Psocoptera				
<i>Echmepteryx hageni</i>	NA	AY630448	HQ124319	AY275298
<i>Liposcelis decolor</i>	NA	AY630472	HQ124320	AY275294
Ambycera				
<i>Austromenopon elliotti</i>	<i>Pelecanoides georgicus</i>	HQ124277	HQ124321	HQ124306
<i>Boopia</i> sp. cf. <i>uncinata</i>	<i>Dasyurus hallucatus</i>	AY077761*	X	X
<i>Colimenopon urocolius</i>	<i>Urocolius indicus</i>	AF385070-1	AF385029	AF385010
<i>Dennyus hirudinis</i>	<i>Apus apus</i>	AF385064-5	AF385032	AF385013
<i>Dennyus dubius</i>	Uncertain	HQ124278	X	X
<i>Dennyus vauxi</i>	Uncertain	HQ124279	X	X
<i>Heterodoxus</i> sp.	<i>Macropus parryi</i>	AY077759*	X	AF270939
<i>Holomenopon</i> sp.	<i>Somateria mollissima</i>	HQ124280	HQ124322	HQ124307
<i>Laemobothrion atrum</i>	<i>Fulica americana</i>	AF385076	AF385035	AF385016
<i>Latumcephalum</i> sp.	<i>Macropus agilis</i>	AY077760*	X	X
<i>Machaerilaemus</i> sp.	<i>Hirudo abyssinica</i>	AF385068-9	AF385031	AF385012
<i>Ricinus</i> sp.	<i>Cyanocompsa parellina</i>	AF385072-3	AF545806	AF545764
<i>Trinoton querquedulae</i>	<i>Anas platyrhynchos</i>	AF385074-5	AF545807	AF385015
Ischnocera				
<i>Acidoproctus emersoni</i>	<i>Dendrocygna eytoni</i>	HQ124281	X	AY314806
<i>Anatoecus</i> sp.	<i>Anas platyrhynchos</i>	AF385056-7	AF385024	AF385006
<i>Austrophilopterus subsimilis</i>	<i>Ramphastos sulphuratus</i>	AF385052-3	AF320365	AF385001
<i>Bovicola</i> sp.	Bovidae	AY077769*	AF320370	AF545680
<i>Campanulotes compar</i>	<i>Columba livia</i>	AF385036-7	AF320377	AF348836
<i>Colilipeurus colius</i>	<i>Urocolius indicus</i>	AF385046-7	AF320381	AF384998
<i>Columbicola columbae</i>	<i>Columba livia</i>	AF385044-5	AF320385	AF278621
<i>Columbicola passerinae</i>	Uncertain	HQ124282	HQ124323	HQ124308
<i>Craspedorrhynchus</i> sp.	<i>Buteo</i> sp.	AY077764-5*	AY314829	AY314811
<i>Degeeriella fulva</i>	<i>Buteo regalis</i>	AY077766*	AF447197	AF444861
<i>Docophoroides brevis</i>	<i>Diomedea epomorpha</i>	HM171418	AF320394	AF396547
<i>Felicola subrostratus</i>	<i>Felis catus</i>	HM171419	AF320398	HM171454
<i>Fulicoffula longipila</i>	<i>Fulica americana</i>	AF385042-3	AF545783	AF545702
<i>Geomydoecus costaricensis</i>	<i>Orthogeomys heterodus</i>	HQ124283	AF320402	L32669
<i>Goniodes</i> sp.	Phasianidae	AY077767*	AF320404	HQ124309
<i>Halipeurus pelagicus</i>	<i>Pelagodroma marina</i>	HQ124284	AY179336	AF396561

<i>Halipeurus procellariae</i>	<i>Pterodroma lessonii</i>	HQ124285	X	AY160051
<i>Naubates</i> sp.	Procellaridae	HQ124286	AY179337	AF396575
<i>Neotrichodectes arizonae</i>	<i>Conepatus leuconotus</i>	HM171420	AF545794	AF545736
<i>Paraclisis diomedea</i>	<i>Thalassarche melanophris</i>	HQ124287	X	HQ124310
<i>Paraclisis hyaline</i>	<i>Diomedea antipodensis</i>	HQ124288	X	AY160041
<i>Pectinopygus brevicornis</i>	<i>Phalacrocorax aristotelis</i>	HQ124289	AF320442	AF497800
<i>Pectinopygus punctatus</i>	<i>Phalacrocorax punctatus</i>	HQ124290	HQ124324	HQ124311
<i>Pectinopygus sulae</i>	<i>Sula sula</i>	AY077768*	AF320444	AY314804
<i>Pectinopygus varius</i>	<i>Phalacrocorax varius</i>	HQ124291	HQ124325	HQ124312
<i>Perineus nigrolimbatus</i>	<i>Fulmarus glacialis</i>	HQ124292	AF320448	AF396589
<i>Stachiella octomaculatus</i>	<i>Procyon</i> sp.	HQ124293	X	X
<i>Struthiolipeurus nandu</i>	<i>Rhea americana</i>	HQ124294	HQ124326	AF545768
<i>Struthiolipeurus stresemanni</i>	<i>Struthio camelus</i>	HQ124295	X	X
<i>Struthiolipeurus struthionis</i>	<i>Struthio camelus</i>	HQ124296	AF320470	AF545769
<i>Trichophilopterus babakotophilus</i>	<i>Propithecus verreaui</i>	HQ124297	AF320476	HQ124313

Rhynchophthirina

<i>Haematomyzus elephants</i>	<i>Elephas maximus</i>	AY077778*	AF320405	AF314816
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Anoplura

<i>Echinophthirius horridus</i> b	<i>Phoca vitulina</i>	HM171377	AF320396	HM171455
<i>Haematopinus phacochoeri</i>	<i>Phacochoerus aethiopicus</i>	AF385058-9	AF385026	AF385007
<i>Haematopinus suis</i>	<i>Sus scrofa</i>	HM171379	EU375777	EU375756
<i>Haematopinus tuberculatus</i>	<i>Bubalus bubalis</i>	HM171380	EU375778	EU375757
<i>Hoplopleura hirsuta</i>	<i>Sigmodon</i> sp.	HQ124298	X	X
<i>Hoplopleura sciuricola</i>	<i>Sciurus carolinensis</i>	HQ124299	EU375783	EU375765
<i>Hoplopleura</i> sp.	<i>Microryzomys altissimus</i>	HQ124300	HQ124327	HQ124314
<i>Hoplopleura trispinosa</i>	<i>Glaucomys volans</i>	HM171395	HM171470	HM171435
<i>Lepidophthirus macrorhini</i>	<i>Mirounga leonia</i>	AY077771*	X	X
<i>Linognathoides marmotae</i>	<i>Marmota</i> sp.	HM171411	EU375780	EU375759
<i>Linognathoides marmotae</i> b	<i>Marmota flaviventer</i>	HQ124301	EU375779	EU375758
<i>Linognathus africanus</i>	<i>Capra hircus</i>	HM171397	X	EU375760
<i>Linognathus ovillus</i>	<i>Ovis aires</i>	HM171398	X	EU375761
<i>Linognathus vituli</i>	<i>Bos taurus</i>	AY077774*	X	X
<i>Neohaematopinus sciuri</i>	<i>Sciurus carolinensis</i>	AF385060-1	AF320433	AF385008
<i>Neohaematopinus sciuri</i> b	<i>Sciurus carolinensis</i>	HQ124302	HQ124328	HQ124315
<i>Neohaematopinus sciurinus</i>	Uncertain	HQ124303	X	X
<i>Pediculus humanus</i> ssp.	<i>Homo sapiens</i>	HQ124304	X	X
<i>Pediculus humanus</i> var <i>capitis</i>	<i>Homo sapiens</i>	AY077775*	HQ124329	HQ124316
<i>Pediculus humanus</i> var <i>capitis</i> b	<i>Homo sapiens</i>	HQ124305	X	HQ124317
<i>Pediculus schaeffi</i>	<i>Pan troglodytes</i>	AY316793	HQ124330	HQ124318
<i>Pedicinus</i> sp.	<i>Trachypothecus pharyrei</i>	AY077777*	X	AY696006
<i>Pthirus pubis</i>	<i>Homo sapiens</i>	AY077776*	X	AY696000
<i>Pterophthirus imitans</i>	<i>Cavia tschudii</i>	HM171396	EU375781	EU375762
