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A phylogenetic test of the Red Queen Hypothesis: Outcrossing and parasitism in the Nematode phylum

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Sexual outcrossing is costly relative to selfing and asexuality, yet it is ubiquitous in nature, a paradox that has long puzzled evolutionary biologists. The Red Queen Hypothesis argues that outcrossing is maintained by antagonistic interactions between host and parasites. Most tests of this hypothesis focus on the maintenance of outcrossing in hosts. The Red Queen makes an additional prediction that parasitic taxa are more likely to be outcrossing than their free-living relatives. We test this prediction in the diverse Nematode phylum using phylogenetic comparative methods to evaluate trait correlations. In support of the Red Queen, we demonstrate a significant correlation between parasitism and outcrossing in this clade. We find that this correlation is driven by animal parasites, for which outcrossing is significantly enriched relative to both free-living and plant parasitic taxa. Finally, we test hypotheses for the evolutionary history underlying the correlation of outcrossing and animal parasitism. Our results demonstrate that selfing and asexuality are significantly less likely to arise on parasitic lineages than on free-living ones. The findings of this study are consistent with the Red Queen Hypothesis. Moreover, they suggest that the maintenance of genetic variation is an important factor in the persistence of parasitic lineages.

KEY WORDS: **Asexual reproduction, continuous-time Markov model, host–parasite coevolution, selfing, sexual outcrossing, stochastic character mapping.**

Outcrossing, the fusion of gametes from two different individuals, is the most prominent reproductive strategy in eukaryotes. Uniparental modes of inheritance, including self-fertilization and parthenogenesis, are in contrast quite rare (Bell 1982; Suomalainen et al. 1987; Dacks and Roger 1999; Billiard et al. 2012). Yet, outcrossing carries significant costs. Theory predicts that these costs accrue as either a significantly depressed per-capita growth rate of outcrossing lineages relative to uniparental lineages or as a reduction in relatedness between parent and offspring (Williams 1975; Maynard Smith 1978; Charlesworth 1980; Lively and Lloyd 1990). A paradox thus emerges: how can the prominence of outcrossing be reconciled with its costs?

The Red Queen Hypothesis offers a potential solution. It proposes that antagonistic coevolution between interacting species selects for the maintenance of outcrossing. If coevolving parasites adapt to specifically infect the most common genotypes in a host population, then rare host genotypes gain a fitness advantage by evading parasitism (Haldane 1949; Jaenike 1978). Outcrossing allows for the production of offspring with rare genotypes, whereas prolonged periods of uniparental reproduction propagate genetically uniform lineages (Hamilton 1980; Hamilton et al. 1990). The Red Queen thus predicts that outcrossing should be maintained in the presence of virulent coevolving parasites. Many empirical studies have supported this prediction by demonstrating that host–parasite coevolution explains the observed distribution of outcrossing in hosts: outcrossing is common in environments or host taxa in which parasite pressure is high (e.g., the tropics, long-lived species; Bell 1982; Burt and Bell 1987; Lively 1987; Verhoeven and Biere 2013; Wilson and Sherman 2013).

Similarly, the Red Queen predicts that outcrossing should be maintained in the coevolving parasites themselves (Bell 1982). Just as hosts are under selection to evade parasitism through the production of rare genotypes, parasites are under equivalent or greater selection to infect their ever-changing host population (Howard and Lively 2002; Galvani et al. 2003; Salathé et al. 2008; King et al. 2011). Parasites continually degrade their environment (the host population) by decreasing the frequency of the common host lineages to which they are adapted. Thus, a common parasite genotype with high fitness is predicted to deplete its host lineage and suffer low fitness in later generations. In contrast, a rare parasite genotype has a greater probability of infecting alternate host genotypes and thus gains a fitness advantage. Few empirical and theoretical studies have investigated this prediction (although see Bell 1982; Howard and Lively 2002; Zhan et al. 2007). A related prediction argues that outcrossing is favored in vertebrate parasites because of selection pressure exerted by the rapidly coevolving adaptive immune system. This prediction has received little support thus far (Gemmil et al. 1997; Lythgoe 2000; West et al. 2001; although see Galvani et al. 2001, 2003).

Bell first formulated the parasite-centric prediction of the Red Queen in his 1982 book *The Masterpiece of Nature*. He argued that under this hypothesis, outcrossing should be more common in parasitic taxa than in their free-living relatives. This prediction was indirectly supported by the difficulty of finding taxa with which to address it: many eukaryotic parasitic groups are invariably outcrossing (e.g., phylum Acanthocephala, subclass Pentastomida; Bell 1982), impeding a comparative approach. Bell (1982) proposed the Nematode phylum as a uniquely diverse taxon for comparative studies. By comparing the reproductive mode and ecology of different nematode families, Bell (1982) offered tentative support for the Red Queen: he found that outcrossing is common in families that parasitize animals, but less so in plant parasitic and free-living families.

A rigorous evaluation of Bell's (1982) prediction that outcrossing is more common in parasitic taxa than in their free-living relatives requires a phylogenetic comparison that accounts for the role of shared ancestry in explaining trait distributions. The tools necessary for this phylogenetic approach were not available at the time of publication of *The Masterpiece of Nature*. Since then, molecular and phylogenetic resources have become available for the Nematoda. Using these tools, studies have identified multiple transitions from free living to parasitism (Blaxter et al. 1998; Dorris et al. 1999; De Ley 2006; van Megen et al. 2009) and from outcrossing to uniparental reproduction in the phylum (Kiontke et al. 2004, 2011; Kiontke and Fitch 2005; Cutter et al. 2008; Denver et al. 2011).

Here, we take advantage of these resources to further test the Red Queen's prediction that outcrossing should be more common in parasitic species than in their free-living relatives. We use a recent phylogeny of the Nematode phylum (Meldal et al. 2007) to make a fine-scale species-level comparison of parasitic and free-living taxa while accounting for shared ancestry. Adding to Bell's preliminary results, we find that the Red Queen Hypothesis successfully explains the macroevolutionary distribution of outcrossing. Although outcrossing is maintained in lineages of parasitic nematodes, notably in animal parasitic lineages, free-living lineages are susceptible to invasion by uniparental modes of reproduction.

Methods

PHYLOGENY

Meldal provided the phylogenetic reconstructions from Meldal et al. (2007), which were based upon small subunit ribosomal DNA for 212 taxa distributed across the phylum. Bayesian inference in MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) produced 2700 trees.

For comparative analyses, we removed the closely related marine clades Desmorida, Chromadorida, and Monhysterida ($n = 48$ taxa). Marine taxa are poorly studied (Meldal et al. 2007), and reproductive mode was difficult to ascertain. An additional two taxa, *Calyptronema maxweberi* and one identified only as a marine Tylenchid, were removed due to lack of character data. Marine nematodes should be the focus of future study: they are thought to be largely outcrossing (Bell 1982).

Pruning in Mesquite version 2.75 (Maddison and Maddison 2011) produced a phylogeny of 162 species with *Turbanella cornuta* as an outgroup. Pruned trees were made ultrametric using maximum-likelihood optimization with the package phangorn version 1.99–5 (Schliep 2011) for R version 3.0.2 (R Core Team 2013). For this purpose, we used the original sequence alignments and the TIM2+I+G model, which was selected in jModelTest under the Bayesian information criterion with the BIONJ setting (Guindon and Gascuel 2003; Darriba et al. 2010).

CHARACTER ASSIGNMENTS

For taxa in their phylogeny, Meldal et al. (2007) reported lifestyle, which is the term used here to distinguish free-living from parasitic taxa. We adopted their lifestyle characterizations, with two exceptions supported by the literature. Reproductive mode was determined through literature reviews, personal communication with experts, and searches of databases (Plant and Insect Parasitic Nematodes [http://nematode.unl.edu/]; Nemaplex [Ferris 1999]; Worm Bazaar [Carter and De Ley 2005]; WormBook [http://www.wormbook.org/chapters/www_quicktourdiversity/quicktourdiversity.html]; Worm Systematic Resource Network [Fitch 1998]). For taxa identified by genus alone, character states were assigned based upon the genus's type or best characterized species.

For reproductive mode, taxa were classified as having an outcrossing or uniparental mode. Uniparental encompasses both parthenogenesis (strict asexuality) and androdioecy (hermaphrodites self-fertilize and occasionally outcross with rare males). For many taxa, male frequency was the primary factor in determining reproductive mode, with rare or absent males indicating uniparental reproduction (Maupas 1900; Triantaphyllou and Hirschmann 1964). Because male rarity cannot distinguish parthenogenesis from androdioecy, these two modes were merged under uniparental reproduction (see Table S1 for details). Theory suggests that the benefits of outcrossing may be obtained by even the rare outcrossing observed for androdioecious nematodes (Hurst and Peck 1996; Agrawal and Lively 2001; Barrière and Félix 2005). Thus, the combination of androdioecy and parthenogenesis is conservative for evaluating our hypothesis.

Ancestral states were estimated with stochastic character mapping (Nielsen 2002; Huelsenbeck et al. 2003) in SIMMAP version 1.5 (Bollback 2006) using the posterior distribution of trees.

CORRELATED EVOLUTION

We used two different phylogenetic comparative methods to test the hypothesis that outcrossing and parasitism are correlated. Each method allows for different analyses, and support for a pattern is greatly strengthened when similar results are obtained using approaches with distinct theoretical frameworks. Firstly, we used stochastic character mapping to measure the correlation between outcrossing and parasitism, a general classification encompassing both plant and animal parasitism. Because this approach allows multistate characters, we also tested the more specific hypothesis that outcrossing and animal parasitism are correlated. Secondly, we used the Discrete method to determine if transitions in lifestyle and reproductive mode are correlated (Pagel 1994). We restricted this analysis to free-living and animal parasitic taxa, excluding plant parasites. This approach estimates transition rates between character states, so we also compared transition rates to test two hypotheses for the observed evolutionary patterns. Basic deviations in character distributions were investigated using chi-square tests in R.

Stochastic character mapping

Stochastic character mapping was implemented in SIMMAP. This method creates stochastic character maps by sampling the posterior distribution of trees and model parameters. Stochastic character maps provide a posterior distribution of character histories with which to quantify character correlation (Nielsen 2002; Huelsenbeck et al. 2003; Bollback 2006). The method measures the observed frequency of co-occurrence of states i and j across character maps and their expected co-occurrence given the frequency of each state. The statistic d is the deviation of observed from expected. Positive values indicate greater co-occurrence than expected, whereas negative values indicate less than expected. Significance is determined by sampling d from character maps constructed under the assumption that character states are not associated. The probability of the observed d value is measured against this null distribution (Huelsenbeck et al. 2003; Bollback 2006).

SIMMAP accounts for uncertainty in modeling character evolution by assigning priors on the parameters for each trait (Schultz and Churchill 1999; Bollback 2006). Prior parameters were obtained using SIMMAP's Markov chain Monte Carlo (MCMC) analysis to generate a posterior distribution for the overall rate of character evolution (under a gamma prior) and the bias parameter (under a beta prior for two-state characters or an empirical prior for three-state characters). Posterior distributions were analyzed in R using the SIMMAP script (http://www.simmap.com/pgs/priors.html) to obtain the best-fitting parameters. Prior parameters were determined independently for each analysis using the consensus phylogeny.

Analyses of character correlation were performed using the 2700 trees, rooted and with outgroup excluded. A total of 100 samples, prior draws, and predictive samples for significance measures were taken. Specific analyses are outlined in Table S3.

Discrete method

The Discrete method (Pagel 1994) was implemented in BayesDiscrete within BayesTraits version 1.0 (Pagel and Meade 2006). BayesDiscrete tests if the evolution of two binary traits is best explained by a model of dependent or independent evolution. Under dependent evolution, transitions in lifestyle depend upon the state of reproductive mode, and vice versa. Under independent evolution, transitions in lifestyle are independent of reproductive mode, and vice versa (Pagel 1994; Pagel and Meade 2006). The Bayesian version allows for two comparisons of competing models (dependent vs. independent). First, we used a Bayes factor of the marginal likelihood of competing models (Kass and Raftery 1995). The marginal likelihood is approximated by the harmonic mean of the likelihoods in a very long Markov chain. Second, we compared the proportion of visits made to independent versus dependent models under the dependent mode of the reversible-jump MCMC. Of the 21,146 models possible under this analysis, 51 (0.24%) are consistent with independent evolution. Therefore, if 0.24% of visits by the reversible-jump MCMC analysis are to independent models, the odds of dependent versus independent models of evolution are equivalent (Pagel and Meade 2006).

Plant parasitic lineages were excluded from the dataset so as to test correlated evolution of animal parasitism and outcrossing. The 2700 trees were rooted and outgroup excluded. Each analysis was run for 100,050,000 iterations, with a burn-in of 50,000 iterations, sampling every 300 iterations. A reversible-jump gamma hyperprior was used, with parameters seeded from uniform distributions on the interval 0–10. A rate deviation parameter of 9 was chosen to obtain an average acceptance rate of 20–40%. Because the harmonic mean of the likelihood may have very large variance and can thus be unstable, five runs of both the independent and dependent analyses were performed (Newton and Raftery 1994; Pagel and Meade 2006; Raftery et al. 2006).

If dependent models of evolution are supported, the underlying transition rate parameters of the dependent analysis can be examined (Pagel and Meade 2006). We tested specific hypotheses by comparing the posterior distributions of the following transition rates: q_{13} with q_{24}, outcrossing to uniparental reproduction on free-living and animal parasitic backgrounds, respectively; q_{13} with q_{12}, free living to parasitism on an outcrossing background; and q_{12} with q_{34}, free living to parasitism on outcrossing and uniparental backgrounds, respectively.

Reversals from uniparental reproduction to outcrossing may be rare, even impossible (Igic et al. 2006; Goldberg and Igic 2008). An additional analysis was therefore conducted with the transition rate from uniparental reproduction to outcrossing (q_{31} and q_{42} in dependent models; beta1 in independent) restricted to 0. Each analysis was run for 1,000,100,000 iterations, with a burn-in of 50,010,000 iterations, sampling every 600 iterations. A gamma hyperprior was used, with parameters seeded from uniform distributions on the interval 0–5. A rate deviation parameter of 5 was chosen. Specific analyses are outlined in Table S4.

Both comparative methods described above rely upon a Markov process that Maddison and FitzJohn (2014) argue is flawed. The crux of the problem lies in the assumption of the Markov process that small branch segments are independent. They particularly cite as problematic datasets in which transitions in a character are rare and/or concentrated in a single lineage. In the supplement, we therefore report the methods and results for estimation of transition numbers. Maddison and FitzJohn (2014) also cite nonrandom sampling of characters as a contributing problem. We address this issue in the supplement by measuring correlations under simulations of different sampling schemes.

Results

ANCESTRAL STATES FOR REPRODUCTIVE MODE AND LIFESTYLE

The phylogeny used in comparative analyses was modified from Meldal et al. (2007) and comprises 162 nematode species (Fig. 1). Character states are summarized in Table 1 and detailed in Table S1. Ancestral states were estimated on the posterior distribution of trees using stochastic character mapping. For lifestyle, a free-living ancestor is strongly supported (probability: 99.5%). For reproductive mode, outcrossing as the ancestral state is weakly supported (probability: 64.2%). We find support for multiple transitions between states for both lifestyle and reproductive mode (Table S2).

CORRELATION BETWEEN OUTCROSSING AND PARASITISM

Stochastic character mapping

In our dataset, the proportion of parasitic species that are outcrossing significantly exceeds that predicted by the joint probabilities of parasitism and outcrossing in the dataset ($\chi^2 = 7.91$, df = 1, $P = 0.005$). This excess of outcrossing holds when accounting for phylogeny: outcrossing and parasitism are significantly positively associated in the evolutionary history of the Nematoda ($d = 0.011$, $P = 0.02$; Table S3).

The excess of outcrossing in parasitic species is driven by animal parasites: 100% are obligate outcrossers ($n = 43$) or have an outcrossing stage in their life cycle ($n = 2$). In contrast, only 60.5% of plant parasites are outcrossing, which is equivalent to the proportion observed in free-living taxa (60.8%; $\chi^2 = 0$, df = 1, $P = 1$). Indeed, a test for correlated evolution contrasting taxa that are free living, parasitic on animals, or parasitic on plants finds that outcrossing is significantly negatively correlated with free living ($d = –0.009$, $P < 0.001$), significantly positively correlated with animal parasitism ($d = 0.011$, $P < 0.001$), and not correlated with plant parasitism ($d = –0.002$, $P = 0.19$). The correlation of outcrossing and animal parasitism is unchanged when 16 species that facultatively associate with animal hosts are treated as animal parasites ($d = 0.010$, $P < 0.001$). The lack of correlation between outcrossing and plant parasitism is similarly unchanged when 14 taxa that are questionably reported as plant parasites are treated as free living ($d = 0.001$, $P = 0.31$). The results are also insensitive to assignment of *Strongyloides ratti* and *Heterorhabditis bacteriophora* as outcrossing or uniparental (Tables 1 and S3).

Discrete method

We then used the Discrete method to determine if evolutionary transitions in lifestyle and reproductive mode are correlated. Given the above results, we excluded plant parasites and limited this analysis to free-living and animal parasitic taxa. This approach further supports correlated evolution of outcrossing and animal parasitism. The estimate of the marginal likelihood of dependent models of evolution, in which evolutionary transitions in lifestyle may depend upon reproductive mode and vice versa, consistently and strongly exceeds that of models of independent evolution (average BF = 14.14; Table S4). The dependent mode of the reversible-jump MCMC analysis can visit both dependent and independent models of character evolution. Yet, this analysis visited independent models less than 0.0001% of the time, which is lower than the 0.24% of visits to independent models predicted if independent and dependent models were equally likely. This further supports dependent, correlated evolution.

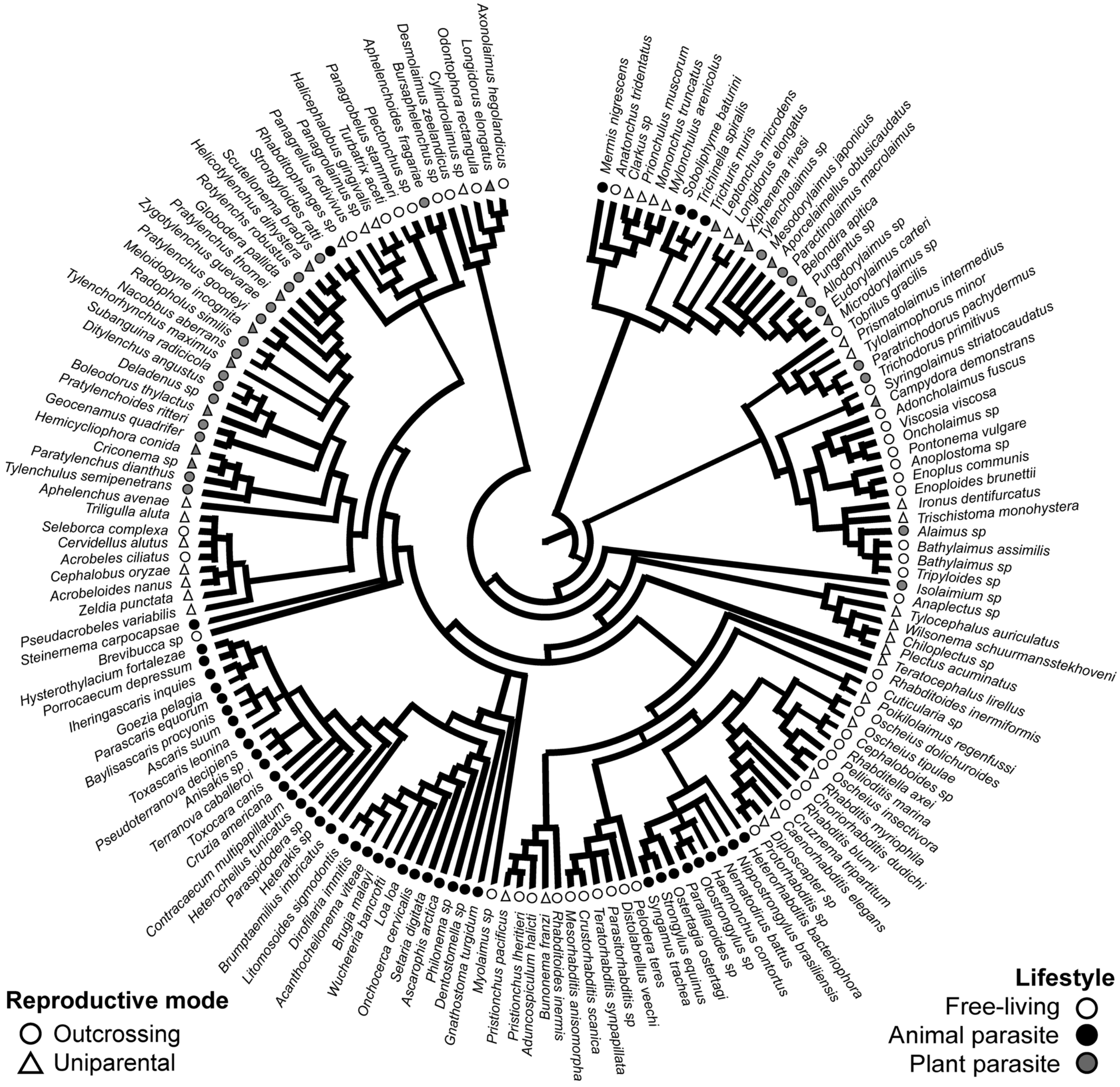

Figure 1. Majority-rule consensus tree and character distribution of 162 species in the Nematoda. This reconstruction represents the consensus of 2700 Bayesian-inferred trees (modified from Meldal et al. 2007). Pruning of the original tree is described in the Methods section. Further modifications were performed in MEGA 5.1 (Tamura et al. 2011). Symbol fill indicates lifestyle: free living (open), animal parasite (black), and plant parasite (gray). Symbol shape indicates reproductive mode: outcrossing (circle) and uniparental (triangle).

EVOLUTIONARY MECHANISMS UNDERLYING THE CORRELATION

We now test two hypotheses for the excess of outcrossing in animal parasites. First, uniparental reproduction may evolve more readily in free-living relative to animal parasitic lineages. Second, animal parasitism may evolve more readily in outcrossing relative to uniparental lineages. Both of these hypotheses are consistent with ancestral state reconstructions here and in prior studies suggesting parasitism and uniparental reproduction as derived states.

Our previous analysis (contrasting free-living and animal parasitic taxa) demonstrates significant support for dependent over independent models of evolution. This allows for further investigation of the dependent models, specifically of the evolutionary transitions underlying correlated evolution. We therefore investigated the transition rate matrix of the dependent

Table 1. Character states for lifestyle and reproduction of 162 nematode species.

		Lifestyle			
		Free living	*Animal parasite*	*Plant parasite*	***Total***
Reproduction	*Outcrossing*	48	45[2]	23	116
	Uniparental	31	0	15	46[4]
	Total	79[1]	45	38[3]	

[1] Sixteen species, identified as free living in Meldal et al. (2007), are reported to have facultative associations with vertebrate ($n = 2$) or invertebrate ($n = 14$) hosts, including parasitism, phoresy, and commensalism. Given the uncertainty regarding the nature of these associations, these taxa are treated as free living unless otherwise noted (Table S1).

[2] Two animal parasites, *Heterorhabditis bacteriophora* and *Strongyloides ratti*, have unusual life cycles in which selfing and parthenogenesis, respectively, typically alternate with biparental outcrossing. These two species are treated as outcrossing unless otherwise noted (Table S1).

[3] Fourteen taxa reported as plant parasites in Meldal et al. (2007) are unlikely to be obligate plant associates. They are commonly reported as soil-dwelling nematodes, in some cases isolated in the vicinity of plant roots. These taxa are treated as plant parasites, to adhere to the reported lifestyle, unless otherwise noted (Table S1).

[4] Fourteen of these uniparental species are parthenogenic, and four are androdioecious. The remaining 28 species are broadly classified as uniparental (Table S1).

models to test our two hypotheses for the observed excess of outcrossing in animal parasites.

Hypothesis 1: Uniparental reproduction evolves more frequently in free-living relative to animal parasitic lineages.

This hypothesis predicts that the transition rate from outcrossing to uniparental reproduction is larger on a free-living (q_{13}) than on an animal parasitic (q_{24}) background (Fig. 2D). Comparison of transition rates under the dependent model of evolution demonstrates significant support for this hypothesis: q_{13} exceeds q_{24} 96.0% of the time, on average, by a large magnitude (average 16.17; Fig. 2A). This result holds when the model is specified to prevent reversals from uniparental reproduction to outcrossing (Table S4). Moreover, the evolution of reproductive mode, rather than of lifestyle, determines the observed evolutionary patterns: transition rates for reproductive mode (q_{13}: outcrossing to uniparental) exceed those for lifestyle (q_{12}: free living to animal parasitism) 96.1% of the time on average (average magnitude 15.57; Fig. 2B). This finding is consistent with Hypothesis 1.

Hypothesis 2: Animal parasitism evolves more frequently in outcrossing relative to uniparental lineages.

This hypothesis predicts that the transition rate from free living to animal parasitism is larger on an outcrossing (q_{12}) than on a uniparental (q_{34}) background. This hypothesis is not supported. Transition rates to animal parasitism on either background are low and statistically indistinguishable ($q_{12} > q_{34}$ 34.7% of the time on average; Fig. 2C).

Discussion

In this study, we test the Red Queen Hypothesis's prediction that outcrossing should be more common in parasitic species than in their free-living relatives. We revisit Bell's (1982) investigation of the distribution of outcrossing and parasitism in the Nematode phylum using phylogenetic comparative tools. The results corroborate Bell's findings: there is a significant positive correlation between outcrossing and parasitism. Also consistent with Bell's results, we find that the relationship between outcrossing and parasitism is limited to animal parasites, with no correlation between plant parasitism and outcrossing. Accordingly, we tested hypotheses for the evolutionary mechanisms generating an excess of outcrossing in animal parasites. Our findings suggest that animal parasitic lineages are more resistant to invasion by uniparental strategies than are free-living lineages. These results strongly support the Red Queen Hypothesis as an explanation for the macroevolutionary distribution of outcrossing in the Nematoda.

Our phylogenetic findings corroborate prior studies of the Red Queen. Of most direct relevance is a theoretical study by Howard and Lively (2002) in which coevolution with hosts maintained outcrossing in parasites, although only in combination with mutation accumulation in clonal parasite lineages. Indirect support also comes from empirical studies of microbial experimental evolution. Coevolution of the bacteria *Bacillus thuringiensis* with nematode hosts resulted in bacterial populations with greater genetic diversity and more frequent horizontal gain of toxin genes, which are likely involved in host interaction (Schulte et al. 2010, 2013). The Red Queen Hypothesis has also been extended to rates of evolution, with the prediction that antagonistic coevolution leads to accelerated molecular evolution (Van Valen 1974; Hedrick 1994; Fischer and Schmid-Hempel 2005; Obbard et al. 2006). Paterson et al. (2010) demonstrated that, relative to phage evolution alone, coevolution of bacteriophage Φ2 with its host, resulted in significantly higher rates of molecular evolution for the phage, most notably at loci

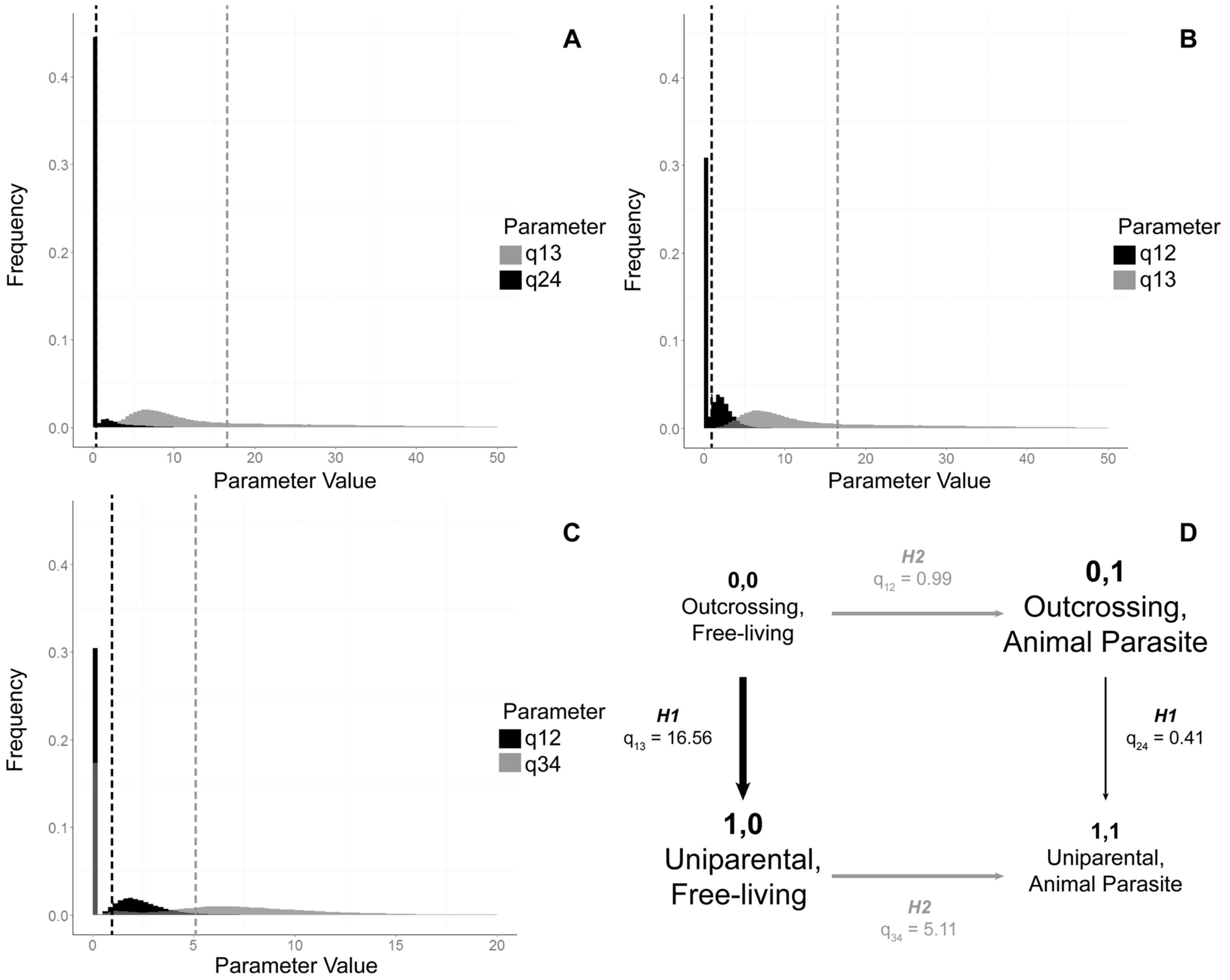

Figure 2. Estimated evolutionary transition rates in reproductive mode and lifestyle. (A–C) Posterior probability distribution of the values of transition rate parameters as estimated in one representative run of five dependent analyses in BayesDiscrete. Comparisons between two different transition rate distributions are displayed to test specific hypotheses. (A) H1: the transition rate from outcrossing to uniparental reproduction is greater on a free living (q_{13}) than on an animal parasitic background (q_{24}). (B) H1: transition rates in reproductive mode (q_{13}) exceed those in lifestyle (q_{12}). (C) H2: the transition rate from free living to animal parasitism is identical on outcrossing (q_{12}) and uniparental backgrounds (q_{34}). Dotted lines indicate the mean estimated transition rate for the corresponding parameter. (D) A diagram of investigated evolutionary transitions between the four different character states for reproductive mode and lifestyle. Larger type indicates character states for which a statistical excess of taxa is found. Line weight corresponds to the magnitude of the transition rate, estimated as the average value across five runs of the dependent analysis in BayesDiscrete. Black indicates transitions compared to test Hypothesis 1 and gray indicates Hypothesis 2.

implicated in host interaction. These microevolutionary results support our macroevolutionary finding that the persistence of parasitic lineages requires forces that maintain genetic variation.

Interestingly, this finding is driven by animal parasites, with plant parasites showing no excess of outcrossing relative to free-living taxa. The occurrence of uniparental reproduction in nematode plant parasites has been previously noted (Triantaphyllou and Hirschmann 1964; Bell 1982; Castagnone-Sereno 2006; Castagnone-Sereno and Danchin 2014). We here propose hypotheses to explain this pattern. First, outcrossing in parasites could be maintained not by coevolution with hosts per se, but rather by coevolution with the adaptive immune systems of vertebrate hosts. Prior studies have not supported this hypothesis (Gemmil et al. 1997; Lythgoe 2000; West et al. 2001), excepting Galvani et al.'s (2003) theoretical demonstration that sexual populations of helminths can resist invasion by asexual mutants. In their study, the advantage of sexual populations stems from their ability to evade host immunity by maintaining strain diversity, which is stochastically lost in asexual parasite populations. Our results are also consistent with this hypothesis: the association of outcrossing and parasitism is present in animal parasites, the vast majority of which parasitize vertebrates, and absent in taxa parasitizing plants. The animal parasites in our dataset that parasitize invertebrates are outcrossing but are too rare ($n = 4$) to provide a valid contrast with vertebrate parasites. Additional sampling of taxa parasitizing invertebrates would allow for a test of the coevolving vertebrate immune system as a force maintaining outcrossing

Second, polyphagous, agricultural pests are overrepresented among nematode plant parasites. Research in scale insects has demonstrated that asexual reproduction is more common in species that are polyphagous and/or pests (Ross et al. 2013). A broad host range may be linked with weak, nonspecific coevolutionary interactions between hosts and parasites that fail to maintain outcrossing (Thompson 1999; Lajeunesse and Forbes 2002). This hypothesis predicts a larger host range for uniparental relative to outcrossing parasites. Alternately, Ross et al. (2013) attribute the relationship between polyphagy, pest status, and uniparental reproduction to population size. Large effective population sizes (N_e) of pest and/or polyphagous species may facilitate the persistence of uniparental lineages by reducing their probability of extinction by various forces (e.g., mutation accumulation, Hill–Robertson effects). Further research is required to properly contrast N_e of uniparental and outcrossing parasitic nematodes (Nadler 1995; Criscione and Blouin 2005).

Hypotheses based upon N_e present alternatives to the Red Queen Hypothesis (Muller 1964; Lynch et al. 1993; Otto and Barton 2001; Keightley and Otto 2006; Otto 2009; Hartfield et al. 2010, 2012), although these are not mutually exclusive (Howard and Lively 1994; Lively and Morran 2014). Indeed, prior theory on the maintenance of outcrossing in parasites argues for a combined role of host–parasite coevolution and forces that characterize finite populations (e.g., mutation accumulation [Howard and Lively 2002], stochastic extinction [Galvani et al. 2003]). Other forces may also influence the distribution of outcrossing in the Nematoda. Although the Red Queen offers an explanation for the short-term maintenance of outcrossing, limited adaptive potential and thus reduced diversification of uniparental lineages may contribute to outrossing's long-term persistence (Fisher 1930; Muller 1932; Maynard Smith 1978; Nunney 1989; Goldberg et al. 2010; de Vienne et al. 2013). Selection for reproductive assurance has also been hypothesized to explain uniparental reproduction in taxa that inhabit unstable environments or disperse widely (e.g., androdioecy in free-living rhabditid nematodes; Baker 1955; Pannell 2002; Weeks et al. 2006).

Our results are consistent with the prediction of the Red Queen, and thus we cannot falsify this major hypothesis for the maintenance of outcrossing. We do acknowledge three future improvements that would test the robustness of our results. First, current tests of correlated evolution cannot account for speciation and extinction rates, which may differ between reproductive modes (Fisher 1930; Muller 1932; Maynard Smith 1978; Nunney 1989; Goldberg et al. 2010). Ignoring this biological reality can result in overestimation of reversals from uniparental reproduction to outcrossing (Maddison 2006; Goldberg and Igic 2008, 2012; Goldberg et al. 2010). We rudimentarily addressed this issue by preventing this reversal in the Discrete analysis, and our results were qualitatively unchanged. Nonetheless, state-dependent diversification should be incorporated when improved phylogenies and comparative tools become available.

Second, Maddison and FitzJohn (2014) have recently argued that comparative methods for measuring correlations of discrete traits are flawed. When transitions in a trait are rare or concentrated in single lineages, a fundamental assumption of these methods is violated. As a result, coincidence may be mistakenly interpreted as correlation. We find support for many dispersed transitions in both reproductive mode and lifestyle, suggesting that our dataset is relatively robust to Maddison and FitzJohn's (2014) methodological concerns. We cannot, however, reject the possibility that the observed associations are detected for reasons other than correlated evolution.

Third, most nematode species remain undescribed (van Megen et al. 2009; Kiontke et al. 2011). Prior studies of continuous traits suggest that under-sampling itself does not inflate estimates of phylogenetic correlation (Freckleton et al. 2002), but that biased sampling can (Ackerly 2000). Meldal et al. (2007) aimed to sample under-represented taxa for their phylogeny, but the sample of terrestrial taxa likely remains biased: first toward parasites, due to their relevance in public health and agriculture (Meldal et al. 2007; van Megen et al. 2009); second toward uniparental taxa, due to their tractability in the laboratory and overrepresentation in temperate regions where sampling has been concentrated (Bell 1982; Igic and Kohn 2006; van Megen et al. 2009). Oversampling of uniparental and/or parasitic taxa produces an underrepresentation of outcrossing, free-living taxa and thus potentially overestimates the correlation of outcrossing and parasitism. We address this issue in the supplement via simulations to compare true measures of correlation to those obtained from biased subsampling. The simulation results argue that the evolutionary correlations reported here are unlikely to be an artifact of biased sampling of the Nematoda. Biased sampling can weakly inflate correlation estimates under stochastic character mapping but not under the Discrete method. Yet, we find here that several unique tests support significant correlated evolution of outcrossing and parasitism (d and m in stochastic character mapping; marginal likelihood and model visitation in and model visitation in BayesDiscrete).

The findings we present argue that the Nematoda is one of the most promising phyla in which to investigate the evolutionary and ecological forces underlying the maintenance of outcrossing. Moreover, the diversity of this group allows for an investigation of the mechanisms promoting genetic variation in parasite populations, a subject of the utmost importance (Grant 1994; Castagnone-Sereno 2002; Galvani et al. 2003; De Meeûs et al. 2009; Castagnone-Sereno and Danchin 2014). Until more complete phylogenies become available, such investigations should focus upon well-studied subgroups within the Nematoda. The genus of root-knot nematodes *Meloidogyne* presents an excellent opportunity to examine variation in reproductive mode within an obligately parasitic group (Castagnone-Sereno and Danchin 2014), whereas clades within the suborders Tylenchina and Rhabditina may be valuable for investigating transitions in lifestyle and reproductive mode at a finer scale (De Ley 2006).

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LITERATURE CITED

Plant and Insect Parasitic Nematodes. Nematology Lab at University of Nebraska Lincoln. Available at http://nematode.unl.edu/.

Ackerly, D. D. 2000. Taxon sampling, correlated evolution, and independent contrasts. Evolution 54:1480–1492.

Agrawal, A. F., and C. M. Lively. 2001. Parasites and the evolution of self-fertilization. Evolution 55:869–879.

Baker, H. G. 1955. Self-compatibility and establishment after 'long-distance' dispersal. Evolution 9:347–349.

Barrière, A., and M. A. Félix. 2005. High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. Curr. Biol. 15:1176–1184.

Bell, G. 1982. The masterpiece of nature: the evolution and genetics of sexuality. University of California Press, Berkeley, CA.

Billiard, S., M. Lopez-Villavicencio, M. E. Hood, and T. Giraud. 2012. Sex, outcrossing and mating types: unsolved questions in fungi and beyond. J. Evol. Biol. 25:1020–1038.

Blaxter, M., P. De Ley, J. Garey, L. Liu, P. Scheldeman, A. Vierstraete, J. Vanfleteren, L. Mackey, M. Dorris, L. Frisse, et al. 1998. A molecular evolutionary framework for the phylum Nematoda. Nature 392:71–75.

Bollback, J. P. 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC Bioinformatics 7:88–94.

Burt, A., and G. Bell. 1987. Mammalian chiasma frequencies as a test of two theories of recombination. Nature 326:803–805.

Carter, K., and P. De Ley. 2005. Paul's Bizarre Worm Bazaar: a selection of fine worms. Available at http://faculty.ucr.edu/˜pdeley/bazaar/bazaarhome.htm.

Castagnone-Sereno, P. 2002. Genetic variability of nematodes: a threat to the durability of plant resistance genes? Euphytica 124:193–199.

———. 2006. Genetic variability and adaptive evolution in parthenogenetic root-knot nematodes. Heredity 96:282–289.

Castagnone-Sereno, P., and E. Danchin. 2014. Parasite success without sex—the nematode experience. J. Evol. Biol. 27:1323–1333.

Charlesworth, B. 1980. The cost of sex in relation to mating system. J. Theor. Biol. 84:655–671.

Criscione, C. D., and M. S. Blouin. 2005. Effective sizes of macroparasite populations: a conceptual model. Trends Parasitol. 21:212–217.

Cutter, A. D., J. D. Wasmuth, and N. L. Washington. 2008. Patterns of molecular evolution in *Caenorhabditis* preclude ancient origins of selfing. Genetics 178:2093–2104.

Dacks, J., and A. J. Roger. 1999. The first sexual lineage and the relevance of facultative sex. J. Mol. Evol. 48:779–783.

Darriba, D., G. Taboada, R. Doalla, and D. Posada. 2010. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9:772.

De Ley, P. 2006 A quick tour of nematode diversity and the backbone of nematode phylogeny. *In* WormBook, ed. The *C. elegans* Research Community, WormBook 1.41.1. Available at http://www.wormbook.org/chapters/www_quicktourdiversity/quicktourdiversity.html

De Meeûs, T., F. Prugnolle, and P. Agnew. 2009. Asexual reproduction in infectious diseases. Pp. 517–533 *in* I. Schön, K. Martens, and P. Dijk, eds. Lost sex. Springer, New York City, NY.

de Vienne, D. M., T. Giraud, and P.-H. Gouyon. 2013. Lineage selection and the maintenance of sex. PLoS One 8:e66906.

Denver, D. R., K. A. Clark, and M. J. Raboin. 2011. Reproductive mode evolution in nematodes: insights from molecular phylogenies and recently discovered species. Mol. Phyl. Evol. 61:584–592.

Dorris, M., P. De Ley, and M. L. Blaxter. 1999. Molecular analysis of nematode diversity and the evolution of parasitism. Parasitol. Today 15: 188–193.

Ferris, H. 1999. The nematode-plant expert information system: a virtual encyclopedia on soil and plant nematodes. University of California, Davis, CA. Available at http://plpnemweb.ucdavis.edu/nemaplex/index.htm.

Fischer, O., and P. Schmid-Hempel. 2005. Selection by parasites may increase host recombination frequency. Biol. Lett. 1:193–195.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford Univ. Press, Oxford, U.K.

Fitch, D. 1998. Worm Systematic Resource Network. Available at http://www.nyu.edu/projects/fitch/WSRN/.

Freckleton, R. P., P. H. Harvey, and M. Pagel. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. Am. Nat. 160: 712–726.

Galvani, A. P., R. M. Coleman, and N. M. Ferguson. 2001. Antigenic diversity and the selective value of sex in parasites. Ann. Zool. Fenn. 38: 305–314.

———. 2003. The maintenance of sex in parasites. Proc. R. Soc. B 270:19–28.

Gemmil, A. W., M. E. Viney, and A. F. Read. 1997. Host immune status determines sexuality in a parasitic nematode. Evolution 51:393–401.

Goldberg, E., and B. Igic. 2008. On phylogenetic tests of irreversible evolution. Evolution 62:2727–2741.

———. 2012. Tempo and mode in plant breeding system evolution. Evolution 66:3701–3709.

Goldberg, E. E., J. R. Kohn, R. Lande, K. A. Robertson, S. A. Smith, and B. Igic. 2010. Species selection maintains self-incompatibility. Science 330:493–495.

Grant, W. N. 1994. Genetic variation in parasitic nematodes and its implications. Int. J. Parasitol 24:821–830.

Guindon, S., and O. Gascuel. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst. Biol. 52:696–704.

Haldane, J. B. S. 1949. Disease and evolution. Ric. Sci. 19:68–76.

Hamilton, W. D. 1980. Sex versus non-sex versus parasite. Oikos 35:282–290.

Hamilton, W. D., R. Axelrod, and R. Tanese. 1990. Sexual reproduction as an adaptation to resist parasites (a review). Proc. Natl. Acad. Sci. USA 87:3566–3573.

Hartfield, M., S. P. Otto, and P. D. Keightley. 2010. The role of advantageous mutations in enhancing the evolution of a recombination modifier. Genetics 184:1153–1164.

———. 2012. The maintenance of obligate sex in finite, structured populations subject to recurrent beneficial and deleterious mutations. Evolution 66:3658–3669.

Hedrick, P. W. 1994. Evolutionary genetics of the major histocompatibility complex. Am. Nat. 143:945–964.

Howard, R. S., and C. M. Lively. 1994. Parasitism, mutation accumulation and the maintenance of sex. Nature 367:554–557.

———. 2002. The ratchet and the Red Queen: the maintenance of sex in parasites. J. Evol. Biol. 15:648–656.

Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Huelsenbeck, J. P., R. Nielsen, and J. P. Bollback. 2003. Stochastic mapping of morphological characters. Syst. Biol. 52:131–158.

Hurst, L. D., and J. R. Peck. 1996. Recent advances in understanding of the evolution and maintenance of sex. Trends Ecol. Evol. 11:46–52.

Igic, B., and J. R. Kohn. 2006. The distribution of plant mating systems: study bias against obligately outcrossing species. Evolution 60:1098–1103.

Igic, B., L. Bohs, and J. R. Kohn. 2006. Ancient polymorphism reveals unidirectional breeding system shifts. Proc. Natl. Acad. Sci. USA 103:1359–1363.

Jaenike, J. 1978. An hypothesis to account for the maintenance of sex within populations. Evol. Theor. 3:191–194.

Kass, R. E., and A. E. Raftery. 1995. Bayes factors. J. Am. Stat. Assoc. 90:773–795.

Keightley, P. D., and S. P. Otto. 2006. Interference among deleterious mutations favours sex and recombination in finite populations. Nature 443:89–92.

King, K. C., J. Jokela, and C. M. Lively. 2011. Trematode parasites infect or die in snail hosts. Biol. Lett. 7:265–268.

Kiontke, K., and D. H. A. Fitch. 2005. The phylogenetic relationships of *Caenorhabditis* and other rhabditids. *In* WormBook, ed. The *C. elegans* Research Community. Available at http://www.wormbook.org/chapters/www_phylogrhabditids/phylorhab.html.

Kiontke, K., N. P. Gavin, Y. Raynes, C. Roehrig, F. Piano, and D. H. Fitch. 2004. *Caenorhabditis* phylogeny predicts convergence of hermaphroditism and extensive intron loss. Proc. Natl. Acad. Sci. USA 101:9003–9008.

Kiontke, K., M. A. Felix, M. Ailion, M. Rockman, C. Braendle, J.-B. Penigault, and D. Fitch. 2011. A phylogeny and molecular barcodes for *Caenorhabditis*, with numerous new species from rotting fruits. BMC Evol. Biol. 11:339–356.

Lajeunesse, M. J., and M. R. Forbes. 2002. Host range and local parasite adaptation. Proc. R. Soc. B 269:703–710.

Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328:519–521.

Lively, C. M., and D. G. Lloyd. 1990. The cost of biparental sex under individual selection. Am. Nat. 135:489–500.

Lively, C. M., and L. T. Morran. 2014. The ecology of sexual reproduction. J. Evol. Biol. 27:1292–1303.

Lynch, M., R. Bürger, D. Butcher, and W. Gabriel. 1993. The mutational meltdown in asexual populations. Heredity 84:339–344.

Lythgoe, K. A. 2000. The coevolution of parasites with host-acquired immunity and the evolution of sex. Evolution 54:1142–1156.

Maddison, W. P. 2006. Confounding asymmetries in evolutionary diversification and character change. Evolution 60:1743–1746.

Maddison, W., and D. Maddison. 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75. Available at http://mesquiteproject.org.

Maddison, W. P., and R. G. FitzJohn. 2014. The unsolved challenge to phylogenetic correlation tests for categorical characters. Syst. Biol. 64:127–136.

Maupas, E. 1900. Modes et formes de reproduction des nematodes. Arch. Zool. Exp. Gen. 3:463–624.

Maynard Smith, J. 1978. The evolution of sex. Cambridge Univ. Press, Cambridge, U.K.

Meldal, B., N. Debenham, P. De Ley, I. De Ley, J. Vanfleteren, A. Vierstraete, W. Bert, G. Borgonie, T. Moens, P. Tyler, et al. 2007. An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. Mol. Phyl. Evol. 42:622–636.

Muller, H. J. 1932. Some genetic aspects of sex. Am. Nat. 66:118–138.

———. 1964. The relation of recombination to mutational advance. Mutat. Res 1:2–9.

Nadler, S. A. 1995. Microevolution and the genetic structure of parasite populations. J. Parasitol 81:395–403.

Newton, M., and A. E. Raftery. 1994. Approximate Bayesian inference with the weighted likelihood bootstrap. J. R. Stat. Soc. B Stat. Methodol. 56:3–48.

Nielsen, R. 2002. Mapping mutations on phylogenies. Syst. Biol. 51:729–739.

Nunney, L. 1989. The maintenance of sex by group selection. Evolution 43:245–257.

Obbard, D. J., F. M. Jiggins, D. L. Halligan, and T. J. Little. 2006. Natural selection drivese extremely rapid evolution in antiviral RNAi genes. Curr. Biol. 16:580–585.

Otto, S. P. 2009. The evolutionary enigma of sex. Am. Nat. 174:S1–S14.

Otto, S. P., and N. H. Barton. 2001. Selection for recombination in small populations. Evolution 55:1921–1931.

Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proc. R. Soc. B 255:37–45.

Pagel, M., and A. Meade. 2006. Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. Am. Nat. 167:808–825.

Pannell, J. R. 2002. The evolution and maintenance of androdioecy. Ann. Rev. Ecol. Syst. 33:397–425.

Paterson, S., T. Vogwill, A. Buckling, R. Benmayor, A. J. Spiers, N. R. Thomson, M. Quail, F. Smith, D. Walker, and B. Libberton. 2010. Antagonistic coevolution accelerates molecular evolution. Nature 464: 275–278.

R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org.

Raftery, A. E., M. A. Newton, J. M. Satagopan, and P. N. Krivitsky. 2006. Estimating the integrated likelihood via posterior simulation using the harmonic mean identity. Working Paper no. 60R, Center for Statistics and Social Sciences. University of Washington, Seattle, WA.

Ross, L., N. B. Hardy, A. Okusu, and B. B. Normark. 2013. Large population size predicts the distribution of asexuality in scale insects. Evolution 67:196–206.

Salathé, M., R. D. Kouyos, R. R. Regoes, and S. Bonhoeffer. 2008. Rapid parasite adaptation drives selection for high recombination rates. Evolution 62:295–300.

Schliep, K. 2011. phangorn: phylogenetic analysis in R. Bioinformatics 27:592–593.

Schulte, R. D., C. Makus, B. Hasert, N. K. Michiels, and H. Schulenburg. 2010. Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. Proc. Natl. Acad. Sci. USA 107:7359–7364.

Schulte, R. D., C. Makus, and H. Schulenburg. 2013. Host-parasite coevolution favours parasite genetic diversity and horizontal gene transfer. J. Evol. Biol. 26:1836–1840.

Schultz, T. R., and G. A. Churchill. 1999. The role of subjectivity in reconstructing ancestral character states: a Bayesian approach to unknown rates, states, and transformation asymmetries. Syst. Biol. 48:651–664.

Suomalainen, E., A. Saura, and J. Lokki. 1987. Cytology and evolution in parthenogenesis. CRC Press, Inc., Boca Raton, FL.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: molecular evolution genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28:2731–2739.

Thompson, J. N. 1999. Specific hypotheses on the geographic mosaic of coevolution. Am. Nat. 153:S1–S14.

Triantaphyllou, A., and H. Hirschmann. 1964. Reproduction in plant and soil nematodes. Ann. Rev. Phytopathol. 2:57–80.

van Megen, H., S. van den Elsen, M. Holterman, G. Karssen, P. Mooyman, T. Bongers, O. Holovachov, J. Bakker, and J. Helder. 2009. A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. Nematology 11:927–950.

Van Valen, L. 1974. Molecular evolution as predicted by natural selection. J. Mol. Evol. 3:89–101.

Verhoeven, K. J. F., and A. Biere. 2013. Geographic parthenogenesis and plant-enemy interactions in the common dandelion. BMC Evol. Biol. 13:23–30.

Weeks, S. C., C. Benvenuto, and S. K. Reed. 2006. When males and hermaphrodites coexist: a review of androdioecy in animals. Integr. Comp. Biol. 46:449–464.

West, S. A., A. W. Gemmil, A. L. Graham, M. E. Viney, and A. F. Read. 2001. Immune stress and facultative sex in a parasitic nematode. J. Evol. Biol. 14:333–337.

Williams, G. C. 1975. Sex and evolution. Princeton Univ. Press, Princeton, NJ.

Wilson, C. G., and P. W. Sherman. 2013. Spatial and temporal escape from fungal parasitism in natural communities of anciently asexual bdelloid rotifers. Proc. R. Soc. B 280:1255–1262.

Zhan, J., C. C. Mundt, and B. A. McDonald. 2007. Sexual reproduction facilitates the adaptation of parasites to antagonistic host environments: evidence from empirical study in the wheat-*Mycosphaerella graminicola* system. Int. J. Parasitol 37:861–870.

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Supporting Information

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Figure S1. Estimates of correlation in subsampled relative to true phylogenies under different sampling schemes.
Table S1. Species-level detail for character assignments.
Table S2. Number of character changes estimated under parsimony and stochastic character mapping.
Table S3. Summary of results of stochastic character mapping.
Table S4. Summary of results of the discrete model for correlated evolution of outcrossing and animal parasitism.
Table S5. Mean and variance of correlation estimates from true and subsampled phylogenies under random and biased sampling.
Table S6. Likelihood ratios and the significance of dependent evolution in true and subsampled phylogenies.

SUPPLEMENTAL MATERIAL

Table S1: Species-level detail for character assignments. Assigned character states for lifestyle and reproductive mode are provided, as well as strain identifiers and alternate nomenclature for the focal species, explanatory details, and the sources used in character assignment.

Species	***Alt. IDs***	***Lifestyle*** ^{a}	***Details*** ^{b}	***Reproduction*** ^{c}	***Details*** ^{d}	***Sources***
Acanthocheilonema viteae		Parasite, animal		Outcrossing		[1, 2]
Acrobeles ciliatus	WCUG2; *A. subcomplexus*	Free-living		Outcrossing		[3-7]
Acrobeloides nanus		Free-living		Uniparental	Parthenogenic	[8, 9]
Adoncholaimus fuscus		Free-living		Outcrossing		[10, 11]
Aduncospiculum halicti		Free-living	Facultative association	Outcrossing		[12]
Alaimus sp.	PDL-2005	Parasite, plant		Outcrossing	Based upon type species, *A. primitivus*	[3, 7, 12, 13]
Allodorylaimus sp.	PDL-2005	Parasite, plant	Free-living	Outcrossing	Based upon type species, *A. uniformis*	[7, 14]
Anaplectus sp.	PDL-2005	Free-living		Outcrossing	Based upon type species, *A. granulosus*	[3, 11, 15]; pers. comm. O Holovachov
Anatonchus sp.	*A. tridentatus*	Free-living		Outcrossing	Based upon type species, *A. tridentatus*	[3, 16, 17]
Anisakis sp.	U81575	Parasite, animal		Outcrossing	Based upon species *A. simplex*	[18, 19]; pers. comm. S D'Amelio
Anoplostoma sp	BHMM-2005	Free-living		Outcrossing	Based upon species *A. viviparum*	[20, 21]
Aphelenchoides fragariae		Parasite, plant	Exception	Outcrossing		[3, 22-24]
Aphelenchus avenae		Free-living		Uniparental	Parthenogenic (meiotic)	[25-29]

Species	***Alt. IDs***	***Lifestyle*** [a]	***Details*** [b]	***Reproduction*** [c]	***Details*** [d]	***Sources***
Aporcelaimellus obtusicaudatus		Parasite, plant	Free-living	Uniparental	Males rare	[30, 31]; pers. comm. MT Vinciguerra
Ascaris suum		Parasite, animal		Outcrossing		[2, 32]
Ascarophis arctica		Parasite, animal		Outcrossing		[33-35] ; pers. comm. G Munoz, R Appy
Ascolaimus elongates		Free-living		Outcrossing		[11, 36, 37]
Axonolaimus hegolandicus		Free-living		Outcrossing		[11, 36-39]
Bathylaimus assimilis		Free-living		Outcrossing	Based upon *B. longicorpus* and *B. anatolii*	[40, 41]
Bathylaimus sp	*B. assimilis*	Free-living		Outcrossing	Based upon *B. longicorpus* and *B. anatolii*	[40, 41]
Baylisascaris procyonis		Parasite, animal		Outcrossing		[42, 43]; Pers. comm. R Overstreet, HP Fagerholm
Belondira apitica		Parasite, plant	Free-living, plant associate	Outcrossing		[7, 44, 45]
Boleodorus thylactus		Parasite, plant	Free-living, plant associate	Uniparental	Males rare	[3, 46, 47]
Brevibucca sp.	SB261	Free-living		Outcrossing		[3, 48]
Brugia malayi		Parasite, animal		Outcrossing		[49-51]
Brumptaemilius imbricatus	Original IDed as *B. justini*	Parasite, animal	Invertebrate host	Outcrossing		[52] pers. comm. D. Hunt
Bunonema franzi		Free-living		Uniparental	Males absent	[53, 54]
Bursaphelenchus sp.		Free-living	Possible facultative association	Outcrossing	Based upon type species, *B. piniperdae*	[3, 55, 56]
Caenorhabditis elegans		Free-living		Uniparental	Androdioecious	[57, 58]
Campydora demonstrans		Parasite, plant	Free-living plant associate	Uniparental	Males rare	[3, 7, 59, 60]

Species	***Alt. IDs***	***Lifestyle*** [a]	***Details*** [b]	***Reproduction*** [c]	***Details*** [d]	***Sources***
Cephaloboides sp.	SB227	Free-living		Outcrossing		[61]
Cephalobus oryzae		Free-living		Uniparental	Parthenogenic	[62, 63]
Cervidellus alutus		Free-living		Uniparental	Parthenogenic	[64]
Chiloplectus sp.	PDL-2005	Free-living		Uniparental	Males rare; Based upon type species *C. andrassyi*	[11, 64, 65]
Choriorhabditis dudichi		Free-living		Outcrossing		[61, 66]
Clarkus sp.		Free-living		Uniparental	Males rare, based upon type species *C. papillatus*	[11, 51, 67] [68]; pers. comm. J. Kotcon
Contracaecum multipapillatum		Parasite, animal		Outcrossing		[2, 69]; Pers. comm. S. D'Amelio
Criconema sp.	PDL2005	Parasite, plant		Uniparental	Males rare; based upon type species *C. giardia*	[3, 70, 71]
Crustorhabditis scanica		Free-living	Facultative association	Outcrossing		[72-74]
Cruzia americana		Parasite, animal		Outcrossing		[2, 75, 76]
Cruznema tripartitum		Free-living	Facultative association	Outcrossing		[61, 66, 77, 78]
Cuticularia sp.	PS2083; *Poikilolaimus*[e]	Free-living		Outcrossing	Based upon type species, *C. oxycerca (P. oxycerca)*[d]	[3, 24, 79, 80]
Cylindrolaimus	202149	Free-living		Uniparental	Males rare; based upon type species *C. communis*	[3, 81]
Deladenus sp.	PDL2005	Parasite, plant	Free-living	Outcrossing		[3, 82]
Dentostomella sp.		Parasite, animal		Outcrossing		[83, 84]; pers. comm. J. Wilkerson

Species	***Alt. IDs***	***Lifestyle*** [a]	***Details*** [b]	***Reproduction*** [c]	***Details*** [d]	***Sources***
Desmolaimus zeelandicus		Free-living		Outcrossing		[3]
Diploscapter sp.	ps1897	Free-living	Possible facultative association	Uniparental	Parthenogenic (mitotic); based upon type species *D. coronatus*	[8, 29, 78, 85]
Dirofilaria immitis		Parasite, animal		Outcrossing		[86, 87]
Distolabrellus veechi		Free-living		Outcrossing		[22, 88, 89]
Ditylenchus angustus		Parasite, plant		Outcrossing		[90]
Enoploides brunettii		Free-living		Outcrossing		[11, 91]
Enoplus communis		Free-living		Outcrossing		[92, 93]
Eudorylaimus carteri		Parasite, plant	Free-living	Outcrossing		[7, 11]
Geocenamus quadrifer		Parasite, plant		Outcrossing		[94]
Globodera pallida		Parasite, plant		Outcrossing		[95]
Gnathostoma turgidum		Parasite, animal		Outcrossing		[96-98]
Goezia pelagia		Parasite, animal		Outcrossing		[99-101] ; pers. comm. S. D'Amelio
Haemonchus contortus		Parasite, animal		Outcrossing		[51, 102]
Halicephalobus gingivalis		Free-living	Facultative association (vertebrates)	Uniparental	Males absent	[103-107]
Helicotylenchus dihystera		Parasite, plant		Uniparental	Parthenogenic (mitotic)	[108, 109]
Hemicycliophora conida		Parasite, plant		Uniparental	Males absent	[3, 110-112]
Heterakis sp.	14690	Parasite, animal		Outcrossing		[113-115]
Heterocheilus tunicatus		Parasite, animal		Outcrossing		[116, 117]

Species	***Alt. IDs***	***Lifestyle*** [a]	***Details*** [b]	***Reproduction*** [c]	***Details*** [d]	***Sources***
Heterorhabditis bacteriophora		Parasite, animal	Invertebrate host	Outcrossing*	Heterogony: coexistence of males, females, and self-fertile hermaphrodites	[118-121]
Hysterothylacium fortalezae		Parasite, animal		Outcrossing		[2, 122, 123]
Iheringascaris inquies		Parasite, animal		Outcrossing		[122, 123]
Ironus dentifurcatus		Free-living		Uniparental	Males rare	[124-127]
Isolaimium	ARK-10 B	Parasite, plant	Free-living plant association	Outcrossing		[3, 7, 128, 129]
Leptonchus microdens		Parasite, plant	Free-living plant associate	Uniparental	Males rare; based upon type species *L. granulosus*	[130, 131]
Litomosoides sigmodontis		Parasite, animal		Outcrossing		[132-134]
Loa loa		Parasite, animal		Outcrossing		[135-137]
Longidorus elongatus		Parasite, plant		Uniparental	Parthenogenic (meiotic)	[138, 139]
Meloidogyne incognita		Parasite, plant		Uniparental	Parthenogenic (mitotic)	[138, 140]
Mermis nigrescens		Parasite, animal	Invertebrate host	Outcrossing		Pers. comm. J. Burr
Mesodorylaimus japonicus		Parasite, plant	Free-living plant associate	Outcrossing	Based on type species *M. mesonyctius*	[141-145] ; pers. comm. R. Peña-Santiago
Mesorhabditis anisomorpha		Free-living		Outcrossing		[61, 118]
Microdorylaimus sp	PDL-2005	Parasite, plant	Free-living plant associate	Uniparental	Males absent	[7, 146, 147]
Mononchus truncatus		Free-living		Uniparental	Males rare	[3, 7, 148-150]

Species	***Alt. IDs***	***Lifestyle*** [a]	***Details*** [b]	***Reproduction*** [c]	***Details*** [d]	***Sources***
Mylonchulus arenicolus		Free-living		Uniparental	Males absent; basedon types species *M. minor*	[3, 151, 152]
Myolaimus sp.	WCUG9, *M. heterurus*	Free-living		Outcrossing	Based upon type *M. heterurus*	[64, 153, 154]
Nacobbus aberrans		Parasite, plant		Outcrossing		[155]
Nematodirus battus		Parasite, animal		Outcrossing		[156, 157] ; pers. comm. J. Van Dijk
Nippostrongylus brasiliensis		Parasite, animal		Outcrossing		[2, 158-160]
Odontophora rectangula		Free-living		Outcrossing		[161-163]
Onchocerca cervicalis		Parasite, animal		Outcrossing	Based on *O. volvulus*	[2, 137, 164-170]
Oncholaimus sp.	BHMM-2005	Free-living		Outcrossing	Based on *O. oxyuris*	[11, 171]
Oscheius dolichuroides		Free-living		Outcrossing		[61, 66, 139]
Oscheius insectivora		Free-living	Facultative association	Outcrossing		[66, 139, 172]
Oscheius tipulae		Free-living	Facultative association	Uniparental	Androdioecious	[51, 66, 139, 171-175]
Ostertagia ostertagi		Parasite, animal		Outcrossing		[176, 177] ; pers. comm. P. Geldhof
Otostrongylus sp.	U81589	Parasite, animal		Outcrossing	Based on *O. circumlitus*	[178]; pers. comm. K. Lehnert
Panagrellus redivivus		Free-living		Outcrossing		[179, 180]
Panagrobelus stammeri		Free-living	Facultative association	Outcrossing		[64, 181]
Panagrolaimus sp.	PS1159	Free-living		Uniparental	Parthenogenic, strain PS1159	[51, 182]
Paractinolaimus macrolaimus		Parasite, plant	Freshwater plant associate	Outcrossing		[3, 183, 184]; pers. comm. MT Vinciguerra

Species	***Alt. IDs***	***Lifestyle*** [a]	***Details*** [b]	***Reproduction*** [c]	***Details*** [d]	***Sources***
Parafilaroides sp.	U81590	Parasite, animal		Outcrossing		[185-188]; pers. comm. PD Dailey
Parascaris equorum		Parasite, animal		Outcrossing		[189, 190]; pers. comm. S. Nadler
Parasitorhabditis sp.	SB281	Free-living	Facultative association	Outcrossing		[191-196]
Paraspidodera sp.	21303	Parasite, animal		Outcrossing	Based on *P. uncinata*	[197]; pers. comm. A. Rossin
Paratrichodorus pachydermus		Parasite, plant		Outcrossing		[198, 199]; pers. comm. W Decraemer
Paratylenchus dianthus		Parasite, plant		Outcrossing		[200-203]
Pellioditis marina		Free-living		Outcrossing		[204, 205]
Pelodera teres		Free-living		Outcrossing		[61, 206, 207]
Philonema sp.	U81574	Parasite, animal		Outcrossing		[208-211]
Plectonchus sp.	PDL0025	Free-living	Facultative association	Outcrossing		[3, 64]
Plectus acuminatus		Free-living		Uniparental	Males absent	[212-215]; pers. comm. O Holovachov
Poikilolaimus regenfussi		Free-living		Uniparental	Males absent	[216]
Pontonema vulgare		Free-living		Outcrossing		[217, 218]
Porrocaecum depressum		Parasite, animal		Outcrossing		[219, 220]; pers. comm. R Overstreet, HP Fagerholm
Pratylenchoides ritteri		Parasite, plant		Outcrossing		[221-223]
Pratylenchus goodeyi		Parasite, plant		Outcrossing		[224-226]
Pratylenchus thornei		Parasite, plant		Uniparental	Males rare	[224, 225]
Prionchulus muscorum		Free-living		Uniparental	Males rare	[11, 227]
Prismatolaimus intermedius		Free-living		Uniparental	Males rare	[11]; pers. comm. A. Zullini
Pristionchus lheritieri		Free-living	Facultative association	Outcrossing		[228-230]

Species	***Alt. IDs***	***Lifestyle*** [a]	***Details*** [b]	***Reproduction*** [c]	***Details*** [d]	***Sources***
Pristionchus pacificus		Free-living	Facultative association	Uniparental	Androdioecious	[51, 229-231]
Protorhabditis sp.	DF5055	Free-living		Outcrossing		[66]
Pseudacrobeles variabilis		Free-living		Uniparental	Parthenogenic	[3, 64]
Pseudoterranova decipiens		Parasite, animal		Outcrossing		[232, 233]
Pungentus sp.	PDL-2005	Parasite, plant	Free-living plant associate	Uniparental	Males absent; based on *P. thornei*	[3, 234]
Radopholus similis		Parasite, plant		Outcrossing		[235]
Rhabditella axei		Free-living		Outcrossing		[66, 236]
Rhabditis blumi	*Metarhabditis blumi*[e]	Free-living	Facultative association (vertebrate)	Outcrossing		[66, 236-239]
Rhabditis myriophila	*Oscheius myriophilus*[e]	Free-living	Facultative association	Uniparental	Androdioecy	[236, 240]; pers. comm. K Kiontke
Rhabditoides inermiformis	SB158, *Haematozoon subulatum*[e]	Free-living		Outcrossing		[66]
Rhabditoides inermis		Free-living		Outcrossing		[66]
Rhabditophanes sp.	KR3021	Free-living	Exception	Uniparental	Parthenogenic	[139, 241, 242]
Rotylenchus robustus		Parasite, plant		Outcrossing		[24, 243, 244]
Scutellonema bradys		Parasite, plant		Outcrossing		[24, 245]
Seleborca complexa		Free-living		Outcrossing		[61, 246, 247]
Setaria digitata		Parasite, animal		Outcrossing		[248, 249]; pers. comm. S McNulty
Soboliphyme baturini		Parasite, animal		Outcrossing		[250-253]; pers. comm. A. Koehler, E Hoberg, M Kinsella
Steinernema carpocapsae		Parasite, animal	Invertebrate host	Outcrossing		[51, 254]

Species	***Alt. IDs***	***Lifestyle*** [a]	***Details*** [b]	***Reproduction*** [c]	***Details*** [d]	***Sources***
Strongyloides ratti		Parasite, animal		Outcrossing*	Heterogamy: alternation of parthenogenic and outcrossing generations	[255, 256]
Strongylus equinus		Parasite, animal		Outcrossing		[257-259]; pers. comm. CR Reinemeyer
Subanguina radicicola		Parasite, plant		Outcrossing		[155, 260, 261]
Syngamus trachea		Parasite, animal		Outcrossing		[2, 262-264]
Syringolaimus striatocaudatus		Free-living		Outcrossing		[265-267]
Teratocephalus lirellus		Free-living		Uniparental	Males rare	[268, 269]; pers. comm. S Bostrom
Teratorhabditis synpapillata		Free-living	Facultative association	Outcrossing		[61, 270, 271]
Terranova caballeroi		Parasite, animal		Outcrossing		[272]; pers. comm. S D'Amelio
Tobrilus gracilis		Free-living		Outcrossing		[273-275]
Toxascaris leonina		Parasite, animal		Outcrossing		[276, 277]
Toxocara canis		Parasite, animal		Outcrossing		[137]; pers. comm. JW Lewis
Trichinella spiralis		Parasite, animal		Outcrossing		[2, 51, 278]
Trichodorus primitivus		Parasite, plant		Outcrossing		[11, 70, 95, 279]; pers. comm. W Decraemer
Trichuris muris		Parasite, animal		Outcrossing		[51, 280]
Triligulla aluta		Free-living		Uniparental	Parthenogenic	[281, 282]
Tripyloides sp.	BHMM-2005	Free-living		Outcrossing		[11, 283, 284]
Trischistoma monohystera		Free-living		Uniparental	Males rare	[11, 24, 285]
Turbatrix aceti		Free-living		Outcrossing		[3, 286]

Species	***Alt. IDs***	***Lifestyle*** [a]	***Details*** [b]	***Reproduction*** [c]	***Details*** [d]	***Sources***
Tylencholaimus sp.	PDL-2005	Parasite, plant	Free-living	Uniparental	Males absent; based upon well-studied *T. parvus*	[287-289]; pers. comm. H. Okada
Tylenchorhynchus maximus		Parasite, plant		Uniparental	Males rare	[7, 290-292]
Tylenchulus semipenetrans		Parasite, plant		Outcrossing		[95, 293]
Tylocephalus auriculatus	PDL0030	Free-living		Uniparental	Males rare	[212, 215, 294]; pers. comm. O Holovachov
Tylolaimophorus minor		Free-living		Uniparental	Males absent	[295]
Viscosia viscosa		Free-living		Outcrossing		[296, 297]
Wilsonema schuurmansstekhoveni		Free-living		Uniparental	Males absent	[215, 298]; pers. comm. O. Holovachov
Wuchereria bancrofti		Parasite, animal		Outcrossing		[299-301]; pers. comm. G Dreyer, W Stolk
Xiphinema rivesi		Parasite, plant		Uniparental	Parthenogenic (meiotic)	[95, 302]
Zeldia punctate		Free-living		Uniparental	Parthenogenic	[3, 8, 64, 303]
Zygotylenchus guevarai		Parasite, plant		Outcrossing		[304, 305]

[a] Lifestyle character states are adopted from [306]. Character states were additionally verified through literature searches, and two changes were made to the lifestyle reported in [306] based upon compelling evidence from the literature.

[b] The two changes to the [306] lifestyle are noted in this column as "Exception." References supporting these exceptions are provided under "Sources." Moreover, the 16 taxa with reports of facultative associations with vertebrate or invertebrate hosts are distinguished by the note "Facultative association." Finally, the 14 taxa whose classification as plant parasites is uncertain are marked with their alternate ecology, either "Free-living", "Free-living plant associate" or "Freshwater plant associate."

[c] Reproductive character states were determined through literature searches. The sources providing evidence for each species' character state are provided under the column "Sources."

[d] The 46 uniparental species are specifically identified as parthenogenic, androdioecious, or having few to no males. Detailed laboratory examination is ultimately required to distinguish parthenogenesis from androdioecy. Many nematode taxa, however, are understudied and undeveloped as laboratory systems. For these taxa, male frequencies aid in distinguishing outcrossing from uniparental reproduction: taxa in which males are rare are unlikely to reproduce exclusively by outcrossing. However, male frequencies cannot separate parthenogenesis from androdioecy. The extreme rarity of males in species known to be androdioecious means that insufficient study effort cannot be excluded as an explanation for the reported absence of males. Additionally, for those species with rare males reported, parthenogenesis may still be possible. We therefore chose to combine androdioecious and parthenogenic species into the category of uniparental

reproduction. Because theory suggests that the benefits of outcrossing may be obtained by even the low frequency of outcrossing observed in androdioecious nematode species [307-309], the combination of androdioecious species and strictly asexual species is a very conservative approach for evaluating our hypothesis.

[e] Sudhaus [118]: 113-178 - suggested changes to classification

* These two species have unique reproductive systems and are analyzed both as outcrossing and uniparental to control for uncertainty.

Multiple transitions in reproductive mode and lifestyle

Maddison and FitzJohn [310] recently argued that character histories in which origins of a trait are rare and/or concentrated in nearby lineages pose particular problems for the phylogenetic comparative methods used in this study. The underlying Markov process assumes that small branch segments are independent. Maddison and FitzJohn suggest that this assumption is inappropriate for such character histories and can lead to mistaken attribution of phylogenetic associations to correlated evolution when coincidence is truly at play. Character histories in which changes are numerous and dispersed should be less susceptible to the issue raised by Maddison and FitzJohn. We therefore estimated the number of changes in each character.

Estimations were performed using parsimony analysis in Mesquite on a consensus tree and in SIMMAP using the posterior distribution of trees. We find support for numerous transitions between states for both traits (Table S2). Moreover, these transitions are dispersed throughout the phylogeny rather than concentrated in single clades (Fig. 1, additional data not shown). These findings are supported by prior work demonstrating multiple independent transitions in lifestyle [311-314] and reproductive mode [315-319].

Given these attributes of our dataset, it is unlikely that the significant associations we observe arise from coincidence and problematic assumptions of the comparative methods used. However, Maddison and FitzJohn [310] emphasize that we do not yet know the extent to which comparative analyses of seemingly robust datasets are compromised by the assumptions of the underlying Markov process. We thus cannot rule out the possibility that the observed associations may be detected for reasons other than correlated evolution.

Table S2: **Number of character changes estimated under parsimony and stochastic character mapping.**

	Reproductive Mode		***Lifestyle***					
Method	*O → U*	*U → O*	*F → AP*	*F → PP*	*AP→F*	*AP→PP*	*PP→F*	*PP→AP*
Parsimony	27	7	6	5	0	1	0	0
Stochastic Character Mapping	308.5	323.4	11.3	11.3	5.4	2.3	6.2	1.2

For the transitions outlined above, “O” indicates outcrossing, “U” uniparental”, “F” free-living, “AP” animal parasite, and “PP” plant parasite.

Table S3: Summary of results of stochastic character mapping. The comparison performed, character states used, and character correlations, as measured in SIMMAP, are provided.

Comparison	***Lifestyle*** [a]	***Reproductive Mode*** [a]	***SIMMAP Correlation*** [b]	
Free-living vs. all parasitic taxa	Table S1, standard	Table S1, standard	F – O P – O	d= -0.011; p= 0.02 d= 0.011 ; p= 0.02
Free-living vs. plant parasitic vs. animal parasitic taxa	Table S1, standard	Table S1, standard	F – O AP – O PP – O	d= -0.009; p<0.001 d= 0.011 ; p<0.001 d= -0.002; p= 0.19
Animal parasitic taxa vs. other (free-living, plant parasitic)	Table S1, standard	Table S1, standard	F – O AP – O	d= -0.016; p<0.001 d= 0.016; p<0.001
Plant parasitic taxa vs. other (free-living, animal parasitic)	Table S1, standard	Table S1, standard	F – O PP – O	d= 0.003 ; p= 0.16 d= -0.003 ; p= 0.16
Free-living vs. plant parasitic vs. animal parasitic taxa	Include 16 facultative taxa as animal parasitic	Table S1, standard	F – O AP – O PP – O	d= -0.008; p= 0.01 d= 0.010; p<0.001 d= -0.002; p= 0.24
Free-living vs. plant parasitic vs. animal parasitic taxa	Treat 14 plant parasitic taxa as free-living	Table S1, standard	F – O AP – O PP – O	d= -0.009; p<0.001 d= 0.008; p<0.001 d= 0.001; p= 0.31
Free-living vs. all parasitic taxa	Table S1, standard	*S. ratti and H. bacteriophora* treated as uniparental	F – O P – O	d= -0.009; p= 0.03 d= 0.009; p= 0.03
Free-living vs. plant parasitic vs. animal parasitic taxa	Table S1, standard	*S. ratti and H. bacteriophora* treated as uniparental	F – O AP – O PP – O	d= -0.008; p<0.001 d= 0.010; p<0.001 d= -0.001; p= 0.21
Free-living vs. plant parasitic vs. animal parasitic taxa	Table S1, standard	Outcrossing and confirmed parthenogenic taxa only[c]	F – O AP – O PP – O	d= 0.003; p=0.49 d= 0.003; p=0.54 d= -0.004; p= 0.37

[a] Indication of “standard” under Lifestyle and Reproductive Mode signifies that the character assignments reported in Table S1 in the corresponding columns were used without exception.

[b] For the correlation statistics obtained through SIMMAP, “F” indicates free-living, “P” parasite, “AP” animal parasite, “PP” plant parasite, and “O” outcrossing.

[c] The correlation of outcrossing and animal parasitism is insignificant when the analysis is restricted to only outcrossing and known parthenogenic taxa, excluding

other uniparental taxa. This is likely due to the rarity of parthenogenesis in the dataset (8.6%).

The statistic *m* is an alternate correlation statistic generated by the stochastic character mapping implemented in SIMMAP. It is similar to *d,* but specifically reports the correlation between character histories. Bollback et al. [320] reports the form of this statistic as:

$$m_{ij} = f_{ij}\log_2\frac{f_{ij}}{f_i f_j}$$

where f_i and f_j indicate the proportion of time that a character history spends in state *i* or *j*, respectively, while f_{ij} indicates the fraction of time that state *i* is associated with state *j* in that character history. Values of the *m* statistic, and measures of its significance, give identical results to the *d* statistics reported above and are thus not reported here.

Table S4: Summary of results of the Discrete model for correlated evolution of outcrossing and animal parasitism. The character states analyzed and any restrictions on transition rate parameters. Five replications of both independent and dependent runs were performed. Independent and dependent runs were compared through a Bayes factor (BF) test and, when in reversible jump MCMC, through the proportion of visits made to independent vs. dependent models. Finally, hypotheses for the evolutionary transitions underlying the observed patterns are tested using the posterior probability that one transition rate parameter exceeds another.

Lifestyle	*Reproductive Mode*	*Uniparental -> Outcrossing*	*BF*	*% Dependent* [a]	*Posterior Probability (value of difference)*		
					H1: $q_{13}>q_{24}$	*H2:* $q_{12}>q_{34}$	$q_{13}>q_{12}$ [b]
Free-living vs. animal parasite	Table S1, standard	Allowed	14.14	>99.99%	96.0% (16.17)	34.7% (-4.10)	96.1% (15.57)
Free-living vs. animal parasite	*S. ratti and H. bacteriophora* treated as uniparental	Allowed	18.23	>99.99%	93.4% (8.22)	<0.01% (-7.4)	99.9% (8.64)
Free-living vs. animal parasite	Table S1, standard	Restricted to zero[c]	3.83	NA	98.1% (2.82)	40.1% (-0.01)	97.7% (2.08)

[a] With the dependent analysis under reversible-jump MCMC, the Markov chain visits a variety of models, 0.24% of which correspond to models of independent trait evolution. If the dependent analysis visits independent models less than 0.24% of the total, dependent trait evolution is supported.

[b] Transition rate parameters correspond to the following transitions: q_{13} – outcrossing to uniparental reproduction, on a free-living background; q_{24} – outcrossing to uniparental reproduction, on an animal parasitic background; q_{12} – free-living to animal parasitism on an outcrossing background; q_{34} – free-living to animal parasitism on a uniparental background.

[c] Reversals from uniparental reproduction to outcrossing may be rare, even impossible. While this hypothesis has not been explicitly tested in nematodes, it is supported by our general knowledge of the diversification rates of uniparental lineages (see Discussion) and by the irreversibility of transitions from self-incompatible to compatible mating systems in angiosperms [321, 322]. To incorporate this biological knowledge in our analyses of correlated evolution, the analyses were conducted again with the probability of reversal from uniparental reproduction to outcrossing (q_{31} and q_{42} in dependent analyses; beta1 in independent analyses) restricted to 0. The reversible-jump MCMC as implemented in BayesDiscrete, which allows comparison of the proportion of visits to dependent and independent models in the dependent analysis, cannot be used with such restrictions, so independent and dependent analyses were only compared with Bayes factor tests. Acceptance rates were extremely low under these restrictions regardless of prior and rate deviation parameters, so the number of iterations was increased to ensure full sampling of the parameter space.

Taxon sampling does not generate positive correlations

The vast majority of taxa in the Nematoda remain undescribed. This fact could complicate the interpretation of our findings of correlated evolution in the main manuscript. In this supplemental section, we therefore test if the observed evolutionary correlation of outcrossing and parasitism could arise merely from the process of under-sampling. In other words, if character states are distributed independently on a “true” phylogeny, can subsampling a small proportion of taxa from the phylogeny generate false positives (type I errors) in tests of evolutionary correlation?

To our knowledge, there are no published tests of the impact of low taxon sampling on contemporary methods for estimating evolutionary correlations of discrete traits. Studies that address this issue for continuous traits suggest that low taxon sampling is unlikely to generate a significant evolutionary correlation that is absent from the true tree. Freckleton et al. [323] find that a measure of phylogenetic correlation, λ, is largely robust to limited phylogenetic information. Moreover, when only a small number of taxa are represented on a phylogeny, the problem that arises is a lack of power to detect correlations that are present, rather than an increase in false positives. Ackerley [324] reports similar findings but additionally emphasizes that nonrandom sampling of taxa can increase the rate of false positives. His finding is intuitive: if subsampling is biased towards selection of taxa in which the character states of interest are both present, then significant correlations between these character states will be found in spite of their absence in the true sample.

There is clearly low taxon sampling of the Nematoda: the true number of taxa is unknown and coarsely estimated to lie between 100 thousand to 100 million [306]. The sub-sampling of these taxa is very likely to have been biased as well. Marine taxa are specifically excluded from this study. Within terrestrial taxa, parasitic taxa are certainly over-represented due to their relevance to human, animal, and crop health [306, 314]. Uniparental taxa are also likely over-represented: they have been historically popular for laboratory use and model system development (e.g. *Caenorhabditis elegans*, *Pristionchus pacificus*). Moreover, most nematode taxa have been sampled from temperate regions [314], where the rates of uniparental reproduction are high relative to the tropics [325, 326]. In reconstructing their phylogeny, Meldal et al. [306] aimed to sample under-represented nematode species from across the phylum, but bias towards uniparental taxa and parasitic taxa undoubtedly remains a significant issue.

Here, we simulate 1000-taxa phylogenies with evolutionary histories in which parasitism and outcrossing are uncorrelated, positively correlated and negatively correlated. We then subsample 100-taxa phylogenies from these true phylogenies under random or biased sampling. Finally, we compare estimates of phylogenetic correlation on the true and subsampled phylogenies. As in our main study, stochastic character mapping and the Discrete method are used to estimate correlation. These methods are implemented in R rather than SIMMAP and BayesDiscrete, respectively, to facilitate replication. From these simulation studies, we conclude that the observation of correlated evolution in our main study is unlikely to have arisen as an artifact of the under-sampling of the Nematoda.

Methods

All simulations were performed in R v3.0.1 [327]. 1000-taxa “true” trees and evolutionary histories were generated in the package phytools v0.4-31 [328]. Two binary traits were investigated in order to represent lifestyle (free-living or parasitic) and reproductive mode (outcrossing or uniparental). We assigned character states to the tips of the true tree by simulating evolutionary histories. To assign tip states under an evolutionary history in which parasitism and outcrossing were not correlated, evolutionary histories of lifestyle and reproductive mode were simulated with independent Q-matrixes. To assign tip states under an evolutionary history in which parasitism and outcrossing were correlated, we simulated two continuous traits with a defined positive or negative correlation. We then applied the function threshState in phytools to convert the tip assignments from continuous to discrete values. This approach consistently generated a non-phylogenetic correlation of outcrossing and parasitism at the tips that was reflective of that specified (data not shown). For each combination of evolutionary history (n=3, no, positive, or negative correlation) and sub-sampling procedure (n=5, see below), five true trees were generated with different Q-matrixes (no correlation) or degrees of correlation (0.7 to 0.9 for positive correlations, -0.7 to -0.9 for negative).

200 100-taxa sub-trees were sub-sampled from each 1000-taxa true tree under five different sampling schemes: random, over-sampling of uniparental taxa, over-sampling of parasitic taxa, over-sampling of outcrossing parasitic taxa, and over-sampling of uniparental and/or parasitic taxa. The first three are self-explanatory. The fourth sampling scheme biased subsampling towards taxa that were both outcrossing and parasitic. This scheme is unlikely to reflect the true bias in sampling of the Nematode phylum: while parasitic taxa are certainly over-represented, there is no evidence that parasitic taxa were additionally sampled according to reproductive mode. We have nonetheless included it to test the hypothesis that specifically sub-sampling taxa in which the traits of interest are present can generate false positive measures of correlation. The fifth sampling scheme is potentially the most representative of the true bias in sampling of the Nematode phylum: taxa were preferentially selected if they were uniparental, parasitic, or both. In other words, taxa that were free-living and outcrossing were specifically under-sampled. In all four biased sampling schemes, favored taxa were twice as likely to be sampled as disfavored taxa.

Due to the extent of replication required, the analyses of simulated phylogenies and associated tip states were not implemented in SIMMAP and BayesDiscrete. We developed our simulations using R packages that implement the same theoretical frameworks, and the general patterns observed should be widely applicable. The correlation of outcrossing and parasitism was tested for five true trees under each combination of evolutionary history and sampling scheme (3 evolutionary histories x 5 sampling schemes = 15 combinations).

To measure the correlation between the character states of outcrossing and parasitism, we used stochastic character mapping [329, 330] implemented in phytools [328]. To analyze a given tree and its associated tip states, we generated one stochastic character map for each trait (reproductive mode and lifestyle). We then determined the expected co-occurrence of outcrossing and parasitism based upon the frequency with which each was independently observed at the nodes of the stochastic character map and the tips of the tree. We also determined the observed co-occurrence based upon the frequency with which outcrossing and parasitism were observed at the same nodes of the stochastic character map and tips of the tree. We define our estimate of correlation as *r:* the observed minus expected frequencies. This value is conceptually similar to the *d* statistic obtained in SIMMAP. We estimated *r* 100 times for each true tree and once on each of 200 subsampled trees. The deviation in *r* was defined as the difference in the mean *r* obtained for the subsampled population minus the mean *r* obtained for the true tree. We averaged these deviations across the five true trees analyzed for each combination of evolutionary history and sampling scheme to assess the overall pattern.

To determine if transitions in lifestyle and reproductive mode were correlated, we used the Discrete method [331] implemented in corHMM v.1.15 [332]. To analyze a given tree and its associated tip states, we estimated the likelihood of a dependent model and an independent model of evolution. We then performed a likelihood ratio test to determine if the dependent model, consistent with correlated evolution, had a significantly higher likelihood than the independent model. We then compared the mean likelihood ratio and mean associated p-value for 100 analyses of each true tree and 200 analyses of the subsampled trees (one analysis per subsampled tree). Finally we averaged these values across the five true trees analyzed for each combination of evolutionary history and sampling scheme to assess the overall pattern.

Results and Discussion

These simulations were performed primarily to address the following question*:* <u>if character states are distributed independently on a “true” phylogeny, is subsampling a small proportion of taxa from the phylogeny likely to generate false</u>

positives in tests of evolutionary correlation? The results of our simulations strongly suggest that the answer to this question is no.

We will first summarize the results when traits are simulated under an evolutionary history of no correlation, beginning with our implementation of stochastic character mapping. Figure S1a demonstrates that the deviation in the estimates of correlation (*r*) of outcrossing and parasitism in subsampled vs. true trees are negligible when subsampling of taxa is random, biased toward uniparental taxa alone, or biased towards parasitic taxa alone. Average estimates of *r* on subsampled trees are slightly higher than those for true trees when subsampling of taxa is biased toward outcrossing parasites or towards taxa that are uniparental and/or parasitic (Fig. S1a,d). The increase in *r* obtained when outcrossing parasites are over-represented in subsamples is intuitive and consistent with prior work [324]. The increase in the estimated correlation of outcrossing and parasitism obtained when uniparental and/or parasitic taxa are over-represented in subsamples is less obvious. It is likely an indirect result of the under-representation of outcrossing, free-living taxa in the subsamples. Given that biased subsampling of uniparental and/or parasitic taxa is likely for the Nematoda, we emphasize the results of this scheme relative to random subsampling.

The variance in estimates of *r* is much higher for the subsampled trees than for the true tree (Fig. S1d, Table S5). This first demonstrates that subsampling does not generate any systematic bias in *r*. We would predict relatively small variances if this were the case. Relatively large variances are consistent with the reduction in phylogenetic information in the subsampled trees relative to the true tree, but not with systematic bias. These large variances nonetheless raise the concern that a tree with a value of *r* consistent with positive phylogenetic correlation could be subsampled from a true tree with no evolutionary history of correlation. If we establish values of *r* greater than or equal to 0.05 as consistent with positive correlation (Fig. S1e), the average percentage of false positives in subsampled trees is quite low, 6.4% under random subsampling and 5.5% under sampling biased towards uniparental and/or parasitic species (Fig. S1d, Table S5).

The results of our implementation of the Discrete method further support the conclusion that subsampling is unlikely to produce false positives in tests for evolutionary correlation. When traits are simulated under an evolutionary history of no correlation, the likelihood ratio tests find no support for the dependent model of evolution on the true trees or subsampled trees under any subsampling scheme (Table S6).

We also mapped traits under the assumptions of positive and negative evolutionary correlations. Subsampling from true trees with evolutionarily correlated traits uniformly diminished our power to detect that correlation. Under both positive and negative correlation, *r* values of subsampled trees were on average closer to zero than those of the true trees (Fig. S1b,c,e,f). Under positive correlations, the deviation was in fact reduced slightly under subsampling biased towards outcrossing parasites or uniparental and/or parasitic taxa (S1b,e). This is consistent with the earlier finding that these sampling schemes can increase the estimated correlation of outcrossing and parasitism. Under negative correlations, the deviation was even closer to zero under these sampling schemes, though the r values remained negative (Fig. S1e,f). Similarly, the likelihood ratio tests consistently found less support for dependent evolution in the subsampled trees relative to the true trees. Indeed, in three of ten cases, dependent evolution was falsely rejected in the subsampled trees (Table S6). These results are consistent with prior findings [323] that subsampling can lead to false negatives in tests of evolutionary correlation due to a loss of power.

Conclusions

The goal of this simulation study was to determine if the evolutionary correlation observed between outcrossing and parasitism in the main study could have arisen merely due to under-sampling of the Nematode phylum. Our tests of both stochastic character mapping and the Discrete method indicate that under-sampling is a very unlikely explanation for the observed positive correlations.

While we are convinced that the general patterns observed in these simulations are applicable to the main study, we acknowledge several differences between the implementations used. Correlation analyses are more rigorous in SIMMAP and BayesDiscrete than in our simulations. The Bayesian approach of SIMMAP and BayesDiscrete allows for weighting of multiple topologies and evolutionary histories. Moreover, in SIMMAP, the correlation statistic *d* is measured across whole topologies, while our *r* statistic is restricted to nodes and tips for logistical reasons. Because *d* accordingly differs from *r* in value, we cannot know if the values of *d* obtained in our main study fall into the region in which false positives might be a concern. However, the values of *d* estimated for the correlation of outcrossing and parasitism are of the same order of magnitude as those obtained in other published tests of correlated evolution implemented in SIMMAP [333-336]. Significance testing is also a powerful component of the SIMMAP and BayesDiscrete implementations that we were unable to replicate in our simulations. In SIMMAP, *d* values obtained for tree and character data are compared to the distribution of *d* values obtained when character states are re-distributed in a random fashion. Under this approach, the *r* values deemed to be false positives in our tests of correlated evolution might well be found to be statistically indistinguishable from those obtained from random trait distributions.

The results of our simulation approach demonstrate that it is very unlikely that the observed correlations of outcrossing and parasitism are an artifact of under-sampling alone. In our main study, we found highly significant support for this correlation in both the stochastic character mapping and Discrete approaches. In our simulations, we never observed simultaneous false positives in these approaches, even under biased subsampling. Moreover, incorporation of the most realistic subsampling scheme for the Nematoda only slightly inflated correlation estimates and did not increase the average rate of false positives. Finally, we find that subsampling can strongly decrease one's power to detect evolutionary correlations present on the true phylogeny and can thus serve as conservative tests of correlation. We therefore conclude that the observed correlation of outcrossing and parasitism in our phylogeny is likely reflective of evolutionary processes in the larger Nematoda.

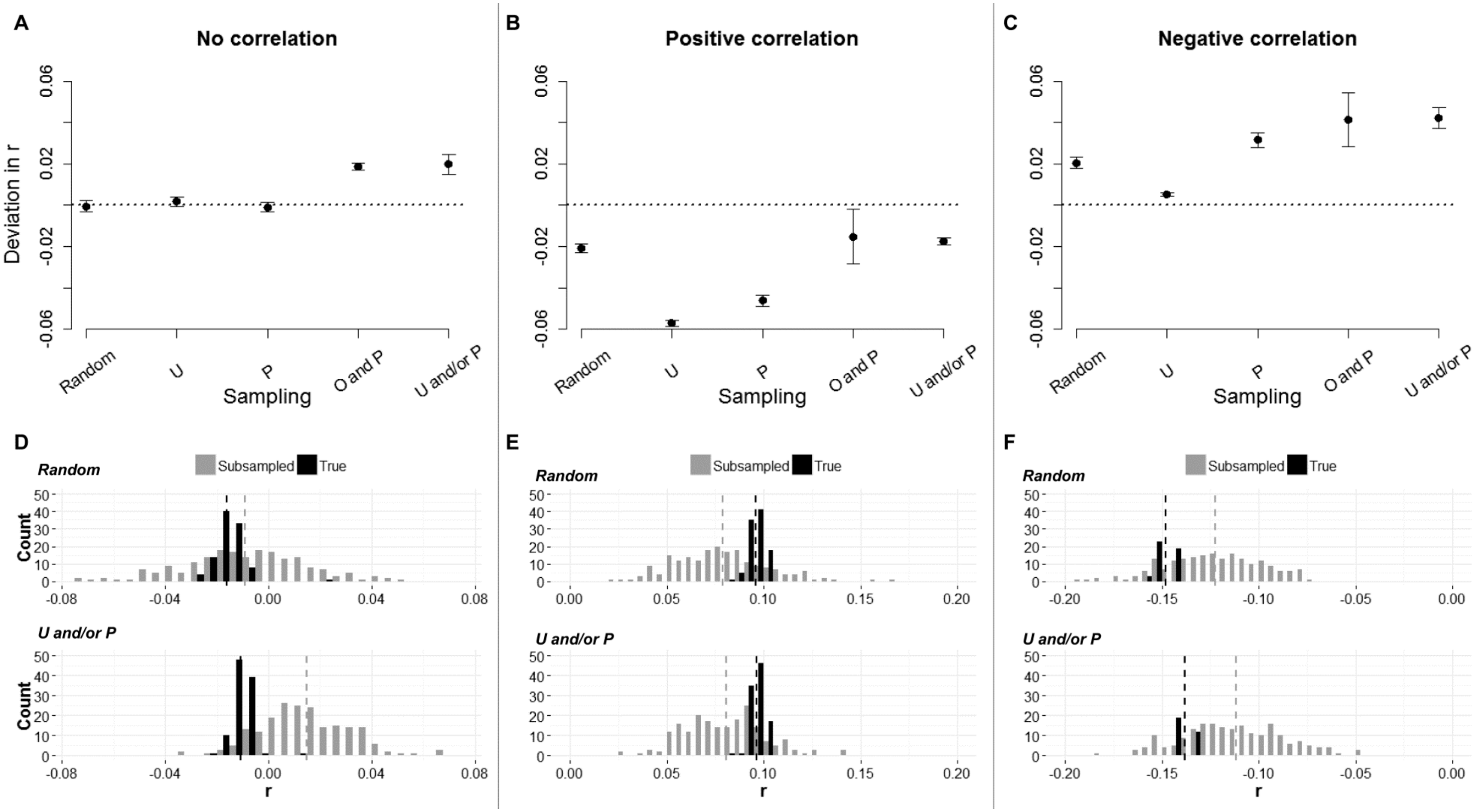

Figure S1: Estimates of correlation in subsampled relative to true phylogenies under different sampling schemes. Trees with 100 taxa were subsampled from true trees with 1000 taxa in which traits were simulated under an evolutionary history with (A,D) no correlation, (B,E) a positive correlation, and (C,F) a negative correlation of outcrossing and parasitism. (A-C) Mean deviations in the correlation estimate *r* from the true phylogeny across different sampling schemes. Deviations were calculated as the difference in the average value of *r* in 200 subsampled trees and the average value of *r* in 100 analyses of the true tree. These deviations were then averaged across five sets of true trees with 200 subsampled trees each. Trees were subsampled from the true tree randomly or under four biased schemes: over-sampling of uniparental taxa (U), of parasitic taxa (P), of outcrossing, parasitic taxa (O and P), or of uniparental and/or parasitic taxa (U and/or P). (D-F) Distribution of *r* values from 100 analyses of a true tree and one analysis of each of its 200 subsampled trees. Representative runs are shown for random sampling and biased sampling favoring uniparental and/or parasitic taxa.

Table S5: Mean and variance of correlation estimates from true and subsampled phylogenies under random and biased sampling. The distributions of outcrossing and parasitism on the true trees were simulated independently (no evolutionary correlation). For each true tree of 1000 taxa, the mean correlation estimate *r* was obtained from 100 analyses. 200 trees of 100 taxa were then subsampled from the true tree. The mean correlation estimate of the subsample was obtained from one analysis per subsample. Subsampled trees were obtained through random sampling of the true tree or biased sampling in favor of uniparental and/or parasitic taxa. The final column indicates the proportion of subsampled trees that might lead to a false positive in a test for correlated evolution of outcrossing and parasitism: *r* exceeded 0.05 though traits were simulated independently.

		True		**Subsample**		
Sampling	*Run*	*r*	*SD*	*r*	*SD*	*proportion r>0.05*
Random	1	-0.016	0.006	-0.009	0.024	0.005
	2	0.008	0.004	0.004	0.017	0
	3	0.040	0.003	0.037	0.023	0.28
	4	-0.004	0.006	0.001	0.024	0.01
	5	0.024	0.004	0.017	0.020	0.025
	Average	**0.010**	**0.005**	**0.010**	**0.022**	**0.064**
U and/or P	1	-0.011	0.003	0.015	0.017	0.025
	2	0.013	0.003	0.019	0.014	0.02
	3	0.016	0.002	0.028	0.017	0.115
	4	-0.012	0.005	0.020	0.023	0.105
	5	-0.010	0.004	0.014	0.016	0.01
	Average	**-0.001**	**0.003**	**0.019**	**0.017**	**0.055**

Table S6: Likelihood ratios and the significance of dependent evolution in true and subsampled phylogenies. The likelihood ratio of dependent vs. independent evolution, and the significance of dependent evolution, was determined 100 times on true trees and once for each of 200 subsampled trees. For each combination of evolutionary history and sampling scheme, the mean likelihood ratio and p-value were estimated for five sets of true and subsampled trees and averaged across the five sets. Traits were simulated according to various correlations of parasitism and outcrossing. For the subsampled trees, taxa were sampled randomly from the true tree or under one of four biased schemes.

			Significance	
Correlation	**Sampling**	*LR Deviation*	*Subsampled*	*Main*
None	Random	0.007814	Not sig.	Not sig.
	U	-1.00152	Not sig.	Not sig.
	P	-0.89961	Not sig.	Not sig.
	O and P	2.144374	Not sig.	Not sig.
	U and/or P	1.058165	Not sig.	Not sig.
Positive	Random	-118.158	Sig.	Highly sig.
	U	-43.5775	Sig.	Highly sig.
	P	-124.02	Sig.	Highly sig.
	O and P	-110.239	Sig.	Highly sig.
	U and/or P	-128.114	Sig.	Highly sig.
Negative	Random	-117.725	Sig.	Highly sig.
	U	-59.4051	Sig.	Highly sig.
	P	-64.6697	Marginal	Highly sig.
	O and P	-86.1672	Not sig.	Highly sig.
	U and/or P	-96.3853	Marginal	Highly sig.

LITERATURE CITED

1. Beaver P, Orihel T, Johnson M: ***Dipetalonema viteae* in the experimentally infected jird, *Meriones unguiculatus*. II. Microfilaremia in relation to worm burden**. *Journal of Parasitology* 1975, **60**(2):310-315.
2. Anderson R: **Nematode Parasites of Vertebrates: Their Development and Transmission**, 2 edn. New York, NY: CABI; 2000.
3. Goodey T: **Soil and Freshwater Nematodes**: Methuen & Co; 1951.
4. Thomas P: **Biology of *Acrobeles complexus* Thorne, cultivated on agar**. *Nematologica* 1965, **11**(3):395-408.
5. Thomas P: **Morphology of *Acrobeles complexus* Thorne cultivated on agar**. *Nematologica* 1965, **11**(3):383-394.
6. Abolafia J, Pena-Santiago R: **Nematodes of the order Rhabditida from Andalucia Oriental, Spain. The genus *Acrobeles* von Linstow, 1877 with description of *A. andalusicus* sp. n. and a key to species**. *Journal of Nematode Morphology and Systematics* 2004, **6**(2):103-128.
7. **Plant and Insect Parasitic Nematodes**. In.: Nematology Lab at University of Nebraska Lincoln; http://nematode.unl.edu/.
8. Heger P, Kroiher M, Ndifon N, Schierenberg E: **Conservation of MAP kinase activity and MSP genes in parthenogenetic nematodes**. *BMC Developmental Biology* 2010, **10**(1):51.
9. Bird A, De Ley P, Bird J: **Morphology, oviposition and embryogenesis in an Australian population of *Acrobeloides nanus***. *Journal of Nematology* 1993, **25**(4):607-615.
10. Calcoen J, Dekegel D: **Spermatozoa in the demanian organ of female *Adoncholaimus fuscus* (Bastian, 1865)(Nematoda)**. *Netherlands Journal of Zoology* 1979, **29**(1):142-143.
11. Abebe E, Traunspurger W, Andrassy I: **Freshwater Nematodes: Ecology and Taxonomy**. Cambridge, MA: CABI; 2006.
12. Giblin RM, Kaya HK: **Associations of halictid bees with the nematodes, *Aduncospiculum halicti* (Diplogasterida: Diplogasteroididae) and *Bursaphelenchus kevini* (Aphelenchida: Aphelenchoididae)**. *Journal of the Kansas Entomological Society* 1984, **57**(1):92-99.
13. Choudhary M, Jairajpuri M: **Six new species of the genus *Alaimus* de Man, 18800 (Nematoda: Alaimidae) from India**. *Revue de Nématologie* 1984, **7**(3):287-300.
14. Andrassy I: **The genus *Eudorylaimus* Andrássy, 1959 and the present status of its species (Nematoda: Qudsianematidae)**. *Opuscula Zoologia Budapest* 1986, **22**:3-42.
15. Holovachov O, Boström S, Winiszewska B, Háněl L: **Description of two known and one new speices of the genus *Anaplectus* De Coninck & Schuurmans Stekhoven, 1993 (Nematoda: Plectida) from Europe, and a revised taxonomy of the genus**. *Russian Journal of Nematology* 2004, **12**(1):45-58.
16. Mulvey RH: **The Mononchidae: a family of predaceous nematodes. II. Genus *Anatonchus* (Enoplida: Mononchidae)**. *Canadian Journal of Zoology* 1961, **39**:807-826.
17. Ahmad W: **Monographs and Perspectives, Volume 7: Mononchida: the Predatory Soil Nematodes**. Boston, MA: Brill Academic Publishers; 2010.

18. Mattiucci S, Nascetti G, Clanchi R, Paggi L, Arduino P, Margolis L, Brattey J, Webb S, D'Amelio S, Orecchia P *et al*: **Genetic and ecological data on the *Anisakis simplex* complex, with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae)**. *The Journal of Parasitology* 1997, **83**(3):401-416.
19. Nascetti G, Paggi L, Orecchia P, Smith JW, Mattiucci S, Bullini L: **Electrophoretic studies on the *Anisakis simplex* complex (Ascaridida: Anisakidae) from the Mediterranean and North-East Atlantic**. *International Journal for Parasitology* 1986, **16**(6):633-640.
20. Surey-Gent S: **Distribution of *Anoplostoma viviparum* (Nematoda) in Southampton water sediments**. *Marine Biology* 1981, **62**:157-160.
21. Tchesunov A, Thanh N: **A desciption of *Anoplostoma nhatrangensis* sp.n. from mangrove habitats of Nha Trang, Central Vietnam, with a review of the genus *Anoplostoma* Bütschli, 1874 (Nematoda: Enoplida)**. *Invertebrate Zoology* 2010, **7**(2):93-105.
22. Jagdale G, Grewal PS: **Infection behavior and overwintering survival of foliar nematodes, *Aphelenchoides fragariae*, on Hosta**. *Journal of Nematology* 2006, **38**(1):130-136.
23. B'Chir M, Dalmasso A: **Meiosis and mitotic chromosome numbers in certain species of the genus *Aphelenchoides***. *Revue de Nématologie* 1979, **2**(2):2469-2257.
24. Ferris H: **The Nematode-Plant Expert Information System: a virtual encyclopedia on soil and plant nematodes**. In*.* http://plpnemweb.ucdavis.edu/nemaplex/index.htm: University of California; 1999.
25. Maupas E: **Modes et formes de reproduction des nematodes**. *Arch Zool Exp Gen* 1900, **3**(8):463-624.
26. Fisher J, Triantaphyllou A: **Observations on development of the gonad and on reproduction in *Aphelenchus avenae***. *Journal of Nematology* 1976, **8**(3):248-255.
27. Fisher J: **Fecundity of *Aphelenchus avenae* Bastian**. *Australian journal of biological sciences* 1968, **21**:169-171.
28. Hansen E, Buecher E, Yarwood E: **Sex differentiatin of *Aphelenchus avenae* in axenic culture**. *Nematologica* 1972, **18**:253-260.
29. Poinar G, Hansen E: **Sex and reproductive modifications in nematodes**. *Helminthological Abstract - Series B* 1983, **52**(4):145-163.
30. Andrassy I: **Two new species of *Aporcelaimellus* (Nematoda: Dorylaimida) from the Americas**. *Acta Zoologica Academiae Scientiarum Hungaricae* 2010, **36**(1):1-8.
31. De Ley P, Loof P, Coomans A: **Terrestrial nematodes from the Galapagos Archipelago II: Redescription of *Aporcelaimellus obtusicaudatus* (Bastian, 1865) Altherr, 1968 with review of similar species and a nomenclature for the vagina in Dorylaimida (Nematoda)**. *Bulletin de L'Institut Royal des Sciences Naturelles de Belgique* 1993, **63**:13-34.
32. Jungersen G, Eriksen L, Nansen P, Fagerholm H: **Sex-manipulated *Ascaris suum* infections in pigs: implications for reproduction**. *Parasitology* 1997, **115**:439-442.
33. Fagerholm H-P, Berland B: **Description of *Ascarophis arctica* Poljansky, 1952 (Nematoda: Cystidicolidae) in Baltic Sea fishes**. *Systematic Parasitology* 1988, **11**(2):151-158.
34. Fagerholm H-P, Butterworth E: ***Ascarophis* sp. (Nematoda: Spirurida) attaining sexual maturity in *Gammarus* spp. (Crustacea)**. *Systematic Parasitology* 1988, **12**(2):123-139.

35. Muñoz G, George-Nascimento M: **Two new specis of *Ascarophis* (Nematoda: Cystidicolidae) in marine fishes from Chile**. *Journal of Parasitology* 2007, **93**(5):1178-1188.
36. Riemann F: ***Nicascolaimus punctatus* gen. et sp. n. (Nematoda, Axonolaimoidea), with notes on sperm dimorphism in free-living marine nematodes**. *Zoologica Scripta* 1986, **15**(2):119-124.
37. Tarjan A, Khuong N: **A compendium of the family Axonolaimidae, (Nematoda)**. *Cahiers de Biologie Marine* 1988, **29**:375-393.
38. Vincx M, Furstenberg JP: ***Namibnema papillata* gen. et sp.n. and *Axonolaimus deconincki* sp.n. (Nematoda, Axonolaimoidea) from marine and estuarine beaches of southern Africa**. *Zoologica Scripta* 1989, **18**(2):231-237.
39. Pavlyuk ON: **Seasonal dynamics of the population density of mass species and trophic groups of free-living marine nematodes**. *Russian Journal of Marine Biology* 2000, **26**(6):399-405.
40. Smirnova E, Fadeeva N: **Description and life cycle of *Bathylaimus anatolii* sp. n. (Nematoda, Tripyloididae)**. *Russian Journal of Marine Biology* 2011, **37**(2):132-142.
41. Keppner E: **Six new species of free-living marine nematodes (Nematoda: Araeolaimida: Enoplida) from two estuaries in northwest Florida, USA**. *Transactions of the American Microscopical Society* 1988, **107**(1):79-95.
42. Kazacos K: ***Baylisascaris procyonis* and related species**. In: *Parasitic Diseases of Wild Mammals.* Edited by Samuel W, Pybus M, Kocan A. Hoboken, NJ: Wiley-Blackwell; 2008: 301-341.
43. Fagerholm H-P: **Systematic implications of male caudal morphology in ascaridoid nematode parasites**. *Systematic Parasitology* 1991, **19**:215-228.
44. Thorne G: **Nematodes of Puerto Rico: Belondiroidea new superfamily, Leptonchidae, Thorne, 1935, and Belonenchidae new family (Nemata, Adenophorea, Dorylaimida)**. *University of Puerto Rico Agricultural Experimental Technical Paper* 1964, **39**:1-51.
45. Naz T, Ahmad W: **Two new and four known species of the genus *Belondira* Thorne, 1939 (Dorylaimida: Belondiridae) from North-Eastern states of India**. *Journal of Nematode Morphology and Systematics* 2009, **12**(2):91-112.
46. Lal A, Khan E: **Nematodes associated with forest trees in northern India-species of Boleodorus Thorne, 1941**. *Myforest* 1988, **24**:301.
47. Geraert E: **Observations on the genera *Boleodorus* and *Boleodoroides* (Nematoda: Tylenchida)**. *Nematologica* 1971, **17**(2):263-276.
48. Yushin VV, Claeys M, Houthoofd W: **Mature spermatozoa of *Brevibucca* sp (Nematoda: Rhabditida: Brevibuccidae)**. *Russian Journal of Nematology* 2011, **19**(2):131-138.
49. Fischer K, Beatty WL, Jiang D, Weil GJ, Fischer PU: **Tissue and stage-specific distribution of *Wolbachia* in *Brugia malayi***. *PLoS Neglected Tropical Diseases* 2011, **5**(5):e1174.
50. Moreno Y, Geary TG: **Stage-and gender-specific proteomic analysis of *Brugia malayi* excretory-secretory products**. *PLoS Neglected Tropical Diseases* 2008, **2**(10):e326.
51. Pires-daSilva A: **Evolution of the control of sexual identity in nematodes**. In: *Seminars in Cell & Developmental Biology: 2007*: Elsevier; 2007: 362-370.

52. Hunt DJ: **The African Carnoyidae (Nematoda: Rhigonematida). 3. Four new species of *Brumptaemilius* Dollfus, 1952**. *Nematology* 2001, **3**(7):627-651.
53. Van Gansbeke R, Bert W, Govaerts A, Claeys M, Van Driessche R, Manhout J, Borgonie G: **Cuticular structure of three *Bunonema* species and first description of the male of *B. penardi* Stefanski, 1914 (Nematoda: Bunonematidae)**. *Nematology* 2004, **6**(4):473-484.
54. Andrassy I: **The genus *Boreolaimus* gen. n. and its six species (Dorylaimida: Qudsianematidae), nematodes from the European Arctic**. *Fundamental and Applied Nematology* 1998, **21**(5):553-568.
55. Kanzaki N, Maehara N, Aikawa T, Togashi K: **First report of parthenogenesis in the genus *Bursaphelenchus* Fuchs, 1937: a description of *Bursaphelenchus okinawaensis* sp. nov. isolated from *Monochamus maruokai* (Coleoptera: Cerambycidae)**. *Zoological science* 2008, **25**(8):861-873.
56. Ryss A, Vieira P, Mota M, Kulinich O: **A synopsis of the genus *Bursaphelenchus* Fuchs, 1937 (Aphelenchida: Parasitaphelenchidae) with keys to species**. *Nematology* 2005, **7**(3):393-458.
57. Stewart AD, Phillips PC: **Selection and maintenance of androdioecy in *Caenorhabditis elegans***. *Genetics* 2002, **160**(3):975-982.
58. Sivasundar A, Hey J: **Sampling from natural populations with RNAi reveals high outcrossing and population structure in *Caenorhabditis elegans***. *Current Biology* 2005, **15**(17):1598-1602.
59. Sturhan D: **On the distribution, ecology and biology of *Campydora demonstrans* Cobb, 1920 (Enoplida, Campydoridae)**. *Nemaologia Mediterranea* 2010, **38**(1):79-82.
60. Jairajpuri M: **Observations on *Campydora* (Nematoda: Dorylaimida)**. *Nematologia Mediterranea* 1983, **11**:33-42.
61. Kiontke K, Fitch D: **New York University Rhabditid Collection Strains List**. In. http://www.nyu.edu/projects/fitch/Rhabditina/NYURC_strainslist.htm.
62. Goldstein B, Frisse LM, Thomas WK: **Embryonic axis specification in nematodes: evolution of the first step in development**. *Current Biology* 1998, **8**(3):157-160.
63. Abolafia J, Pena-Santiago R: **Nematodes of the order Rhabditida from Andalucia Oriental, Spain. The genus *Cephalobus* Bastian, 1865 with description of *C. harpagonis* sp. n. and key to species**. *Nematology* 2009, **11**(4):485-508.
64. Carter K, De Ley P: **Paul's Bizarre Worm Bazaar: a selection of fine worms**. In. http://faculty.ucr.edu/~pdeley/bazaar/bazaarhome.htm; 2005.
65. Steiner W: **The male of *Chiloplectus andrassyi* (Timm, 1971) Andrassy, 1984 (Nematoda, Plectidae)**. *Revue de Nématologie* 1987, **10**(2):247-248.
66. Kiontke K, Fitch D: **The phylogenetic relationships of *Caenorhabditis* and other rhabditids**. In: *WormBook.* Ed. the *C. elegans* Research Community: doi/10.1895/wormbook.1.11.1, http://www.wormbook.org.; 2005.
67. Salinas KA, Kotcon J: **In vitro culturing of the predatory soil nematode *Clarkus papillatus***. *Nematology* 2005, **7**(1):5-10.
68. Salinas KA: **The biology and ecology of interactions between the predatory nematode *Clarkus papillatus* Bastian and selected bacterial biological control agents**. Morgantown, West Virginia: West Virginia University; 2003.

69. Lucker J: **A redescription of *Contracaecum multipapillatum* (Von Drasche, 1882) (Nematoda: Anisakinae)**. *The Journal of Parasitology* 1941, **27**(6):505-512.
70. Triantaphyllou A, Hirschmann H: **Reproduction in plant and soil nematodes**. *Ann Rev Phytopath* 1964, **2**(1):57-80.
71. Raski DJ, Luc M: **A reappraisal of the genus *Criconema* Hofmanner & Menzel, 1914 (Nematoda: Criconematidae)**. *Revue de Nématologie* 1984, **7**:323-334.
72. Bhatti M, Tabassum K, Firoza K: **New records of Mesorhabditidae (Nematoda: Rhabditida) from different habitats of Sindh and Punjab, Pakistan**. *Pakistan Journal of Nematology* 2011, **29**(2):119-137.
73. Adamson M, Rigby M: ***Rhabditis (Crustorhabditis) stasileonovh*(Belugov) from beach hoppers (Talitridae; Amphipoda) from the Pacific Coast of North America**. *Fundamental and Applied Nematology* 1996, **19**(6):579-584.
74. Nicholas WL: ***Crustorhabditis chitwoodi* sp. nov.(Nematoda: Rhabditidae): an intertidal species from the coast of New South Wales, Australia, with observations on its ecology and life history**. *New Zealand Journal of Marine and Freshwater Research* 2004, **38**(5):803-808.
75. Crites JL: **A redescription of *Cruzia americana*, a nematode parasitic in the opossum, *Didelphis marsupialis virginiana***. *Journal of Parasitology* 1956, **42**(1):68-72.
76. Adnet F, Anjos D, Menezes-Oliveira A, Lanfredi R: **Further description of *Cruzia tentaculata* (Rudolphi, 1819) Travassos, 1917 (Nematoda: Cruzidae) by light and scanning electron microscopy**. *Parasitology Research* 2009, **104**(5):1207-1211.
77. Ferris H, Eyre M, Venette R, Lau S: **Population energetics of bacterial-feeding nematodes: stage-specific development and fecundity rates**. *Soil Biology and Biochemistry* 1996, **28**(3):271-280.
78. Warburton EM, Zelmer DA: **Prerequisites for parasitism in rhabditid nematodes**. 2010.
79. Khan E, Singh M, Baird S: **Studies on rhabditids of India: descriptions of *Flagicaudoides* gen. n. and two new species of *Cuticularia* (Nematoda: Rhabditida)**. *International Journal of Nematology* 1999, **9**(2):196-202.
80. Andrassy I: **A taxonomic review of the suborder Rhabditina (Nematoda: Secernentia)**. Paris, France: ORSTOM; 1983.
81. Cobb N: **Revision of the genus *Cylinrolaimus***. *Journal of Parasitology* 1961, **2**:199-200.
82. Wood F: **Biology of *Deladenus durus* (Nematoda: Tylenchida)**. *N Z J Zool* 1974, **1**(1):51-58.
83. Adamson M: **Haplodiploidy in the Oxyurida: decoupling the evolutionary processes of adaptation and speciation**. *Annales de Parasitologie Humaine et Comparée* 1990, **65**(Supplement I):31-35.
84. Adamson ML: **Evolutionary biology of the Oxyurida (Nematoda): biofacies of a haplodiploid taxon**. *Advances in Parasitology* 1989, **28**:175-228.
85. Lahl V, Schulze J, Schierenberg E: **Differences in embryonic pattern formation between *Caenorhabditis elegans* and its close parthenogenetic relative *Diploscapter coronatus***. *International Journal of Developmental Biology* 2009, **53**(4):507.
86. Sakaguchi Y, Kihara S, Tada I: **The chromosomes and gametogenesis of *Dirofilaria immitis***. *Japanese Journal of Parasitology* 1980, **29**(5):377-381.
87. Lok J, Knight D, Selavka C, Eynard J, Zhang Y, Bergman R: **Studies of reproductive competence in male *Dirofilaria immitis* treated with milbemycin oxime**. *Tropical Medicine and Parasitology* 1995, **46**(4):235-240.
88. Doucet M, de Doucet M: **Contribution to the knowledge of *Distolabrellus veechi* Anderson, 1983 (Nemata: Rhabditida)**. *Fundamental and Applied Nematology* 1992, **15**(5):413-420.

89. Anderson R: **Description of *Distolabrellus veechi* n. gen., n. sp. (Nematoda: Rhabditidae)**. *Journal of Nematology* 1983, **15**(1):70--75.
90. Das D, Bajaj HK: **Redescription of *Ditylenchus angustus* (Butler, 1913) Filipjev, 1936**. *Annals of Plant Protection Sciences* 2008, **16**(1):195-197.
91. Benwell M: **A new species of *Gonionchus* (Nematoda: Xyalidae) from the Firth of Clyde, with a redescription of *Enoploides spiculohamatus* Schulz (Nematoda: Enoplidae)**. *Cahiers de Biologie Marine* 1981, **22**(2).
92. Wieser W, Kanwisher J: **Growth and metabolism in a marine nematode, *Enoplus communis* Bastian**. *Zeitschrift für vergleichende Physiologie* 1960, **43**(1):29-36.
93. Atkinson H: **The respiratory physiology of the marine nematodes *Enoplus brevis* (Bastian) and *E. communis* (Bastian) I. The influence of oxygen tension and body size**. *Journal of Experimental Biology* 1973, **59**(1):255-266.
94. Brzeski M: **Taxonomy of *Geocenamus* Thorne Malek, 1968 (Nematoda: Belonolaimidae)**. *Nematologica* 1991, **37**:125-173.
95. Castagnone-Sereno P: **Genetic variability of nematodes: a threat to the durability of plant resistance genes?** *Euphytica* 2002, **124**(2):193-199.
96. Nawa Y, de la Cruz-Otero MC, Zazueta-Ramos ML, Bojórquez-Contreras A, Sicairos-Félix J, Campista-León S, Torres-Montoya EH, Sánchez-Gonzales S, Guzmán-Loreto R, Delgado-Vargas F: **Is *Gnathostoma turgidum* an annual parasite of opossums? Drastic seasonal changes of infection in *Didelphis virginiana* in Mexico**. *Journal of Parasitology* 2009, **95**(4):908-912.
97. Díaz-Camacho SP, Delgado-Vargas F, Willms K, del Carmen de la Cruz-Otero M, Guadalupe Rendón-Maldonado J, Robert L, Antuna S, Nawa Y: **Intrahepatic growth and maturation of *Gnathostoma turgidum* in the natural definitive opossum host, *Didelphis virginiana***. *Parasitology International* 2010, **59**(3):338-343.
98. Miyazaki I: **On the genus *Gnathostoma* and human gnathostomiasis, with special reference to Japan**. *Expermintal Parasitology* 1960, **9**(3):338-370.
99. Martins M, Yoshitoshi E: **A new nematode species *Goezia leporini* n. sp.(Anisakidae) from cultured freshwater fish *Leporinus macrocephalus* (Anostomidae) in Brazil**. *Brazilian Journal of Biology* 2003, **63**(3):497-505.
100. Rasheed S: **On a remarkable new nematode, *Lappetascaris lutjani* gen. et sp. nov.(Anisakidae: Ascaridoidea) from marine fishes of Karachi and an account of *Thynnascaris inquies* (Linton, 1901) n. comb. and *Goezia intermedia* n. sp**. *Journal of Helminthology* 1965, **39**(04):313-342.
101. Deardorff TL, Overstreet RM: **Taxonomy and biology of North American species of *Goezia* (Nematoda: Anisakidae) from fishes, including three new species**. *Proceedings of the Helminthological Society of Washington* 1980, **47**(2):192-217.
102. Redman E, Grillo V, Saunders G, Packard E, Jackson F, Berriman M, Gilleard JS: **Genetics of mating and sex determination in the parasitic nematode *Haemonchus contortus***. *Genetics* 2008, **180**(4):1877-1887.
103. Fonderie P, Bert W, Hendrickx F, Houthoofd W, Moens T: **Anthelmintic tolerance in free-living and facultative parasitic isolates of *Halicephalobus* (Panagrolaimidae)**. *Parasitology-Cambridge* 2012, **139**(10):1301.

104. Nadler SA, Carreno RA, Adams BJ, Kinde H, Baldwin JG, Mundo-Ocampo M: **Molecular phylogenetics and diagnosis of soil and clinical isolates of *Halicephalobus gingivalis* (Nematoda: Cephalobina: Panagrolaimoidea), an opportunistic pathogen of horses**. *International Journal for Parasitology* 2003, **33**(10):1115-1125.
105. Isaza R, Schiller CA, Stover J, Smith PJ, Greiner EC: ***Halicephalobus gingivalis* (Nematoda) infection in a Grevy's zebra (*Equus grevyi*)**. *Journal of Zoo and Wildlife Medicine* 2000, **31**(1):77-81.
106. Houthoofd W, Borgonie G: **The embryonic cell lineage of the nematode *Halicephalobus gingivalis* (Nematoda: Cephalobina: Panagrolaimoidea)**. *Nematology* 2007, **9**(4):573-584.
107. Akagami M, Shibahara T, Yoshiga T, Tanaka N, Yaguchi Y, Onuki T, Kondo T, Yamanaka T, Kubo M: **Granulomatous nephritis and meningoencephalomyelitis caused by *Halicephalobus gingivalis* in a pony gelding**. *Journal of Veterinary Medical Science* 2007, **69**(11):1187-1190.
108. Triantaphyllou A, Hirschmann H: **Cytology and reproduction of *Helicotylenchus dihystera* and *H. erythrinae*** *Nematologica* 1967, **13**(4):575-580.
109. Hirschmann H, Triantaphyllou A: **Mode of reproduction and development of the reproductive system of *Helicotylenchus dihystera***. *Nematologica* 1967, **13**(4):558-574.
110. Brzeski M, Ivanova T: **Taxonomic notes on *Hemicycliophora* de Man (Nematoda: Hemicycliophoridae)**. *Nematologia Mediterranea* 1978, **6**:147-162.
111. Castillo P, Gomez-Barcina A, Loof P: **On two species of *Hemicycliophora* De Man, 1921 (Nematoda: Criconematoidea) found in Spain**. *Nematologia Mediterranea* 1989, **17**:77-82.
112. Monteiro A, Lordello L: **A description of *Hemicycliophora poranga* N. sp., from Brazil (Nemata)**: ESALQ,[19--]; 1978.
113. Walton AC: **Studies on nematode gametogenesis**. *Zeitschrift für Zellen-und Gewebelehre* 1924, **1**(2):167-239.
114. Lund EE, Chute AM, Wilkins G: **The wild turkey as a host for *Heterakis gallinarum* and *Histomonas meleagridis***. *Journal of Wildlife Diseases* 1975, **11**(3):376-381.
115. Lee D, Lešťan P: **Oogenesis and egg shell formation in *Heterakis gallinarum* (Nematoda)**. *Journal of Zoology* 1971, **164**(2):189-196.
116. Sprent J: **Ascaridoid nematodes of amphibians and reptiles: *Typhlophorus, Hartwichia* and *Trispiculascaris***. *Journal of Helminthology* 1983, **57**(02):179-189.
117. Sprent J: **Ascaridoid nematodes of sirenians -the Heterocheilinae redefined**. *Journal of Helminthology* 1980, **54**:309-327.
118. Sudhaus W: **Phylogenetic systematisation and catalogue of paraphyletic" Rhabditidae"(Secernentea, Nematoda)**. *Journal of Nematode Morphology and Systematics* 2011, **14**(2):113-178.
119. Koltai H, Glazer I, Segal D: **Reproduction of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar, 1976: hermaphroditism vs amphimixis**. *Fundamental and Applied Nematology* 1995, **18**(1):55-61.
120. Kahel-Raifer H, Glazer I: **Environmental factors affecting sexual differentiation in the entomopathogenic nematode *Heterorhabditis bacteriophora***. *Journal of Experimental Zoology* 2000, **287**(2):158-166.
121. Ciche T: **The biology and genome of *Heterorhabditis bacteriophora***. In: *WormBook.* doi/10.1895/wormbook.1.135.1, http://www.wormbook.org.: Ed. the *C. elegans* Research Community; 2007.

122. Bruce NL, Cannon LR: ***Hysterothylacium, Iheringascaris* and *Maricostula* new genus, nematodes (Ascaridoidea) from Australian pelagic marine fishes**. *Journal of Natural History* 1989, **23**(6):1397-1441.
123. Deardorff TL, Overstreet RM: **Review of *Hysterothylacium* and *Iheringascaris* (both previously= *Thynnascaris*)(Nematoda: Anisakidae) from the northern Gulf of Mexico**. *Proceedings of the Biological Society of Washington* 1980, **93**(4):1035-1079.
124. Argo A, Heyns J: **Four new species of the genus *Ironus* Bastian, 1865 (Nematoda: Ironidae) from South Africa**. *Phytophylactica* 1972, **4**:59-66.
125. Ebsary B: **Two new aquatic species of *Ironus* Bastian, 1865 (Nematoda: Ironidae) from Canada**. *Canadian Journal of Zoology* 1985, **63**(6):1368-1370.
126. Loof P: **Freshwater nematodes from Suriname collected by J. van der Land**. *Zoologische Verhandelingen* 1973, **129**(1):1-46.
127. Tahseen Q, Mehdi S: **Taxonomy and relationships of a new and the first continental species of *Trissonchulus* Cobb, 1920 along with two species of *Ironus* (Nematoda: Ironidae) collected from coal mines**. *Nematologia Mediterranea* 2009, **37**(2):117-132.
128. Timm R: **The genus *Isolaimium* Cobb, 1920 (Order Isolaimida, Isolaimiidae New Family)**. *Journal of Nematology* 1969, **1**(2):97.
129. Timm R: **The systematic position of *Isolaimium* Cobb, 1920 (Nematoda), with a description of a new species**. *Journal of the Bombay Natural History Society* 1961, **58**:300-303.
130. Loof P: **A review of the nematode genus *Leptonchus* (Enoplida)**. *Nematologica* 1963, **9**(4):507-520.
131. Goseco C, Ferris VR, Ferris JM: **Revisions in Leptonchoidea (Nematoda: Dorylaimida). *Leptonchus, Proleptonchus, Funaria,* and *Meylis* n. gen. in Leptonchidae, Leptonchinae**. *Research Bulletin, Agricultural Experiment Station, Purdue University, West Lafayette* 1974(911).
132. WEBBER W: **The reproductive system of *Litomosoides carinii*, a filarial parasite of the cotton rat. II. The frequency of insemination**. *Annals of Tropical Medicine and Parasitology* 1954, **48**(4):375.
133. Mössinger J, Wenk P: **Fecundity of *Litomosoides carinii* (Nematoda, Filarioidea) in vivo and in vitro**. *Zeitschrift für Parasitenkunde* 1986, **72**(1):121-131.
134. WA W: **The reproductive system of *Litomosoides carinii,* a filarial parasite of the cotton rat. I. Development of gonads and initial insemination**. *Annals of Tropical Medicine and Parasitology* 1954, **48**(4):367-374.
135. Post R, Pinder M: **Oogenesis and embryogenesis in *Loa loa***. *Journal of Helminthology* 1995, **69**(04):351-356.
136. Boussinesq M: **Loiasis**. *Annals of Tropical Medicine and Parasitology* 2006, **100**(8):715-731.
137. De Meeûs T, Prugnolle F, Agnew P: **Asexual reproduction in infectious diseases**. In: *Lost Sex.* Springer; 2009: 517-533.
138. Castagnone-Sereno P: **Genetic variability and adaptive evolution in parthenogenetic root-knot nematodes**. *Heredity* 2006, **96**(4):282-289.
139. Denver D, Clark K, Raboin M: **Reproductive mode evolution in nematodes: insights from molecular phylogenies and recently discovered species**. *Molecular Phylogenetics and Evolution* 2011, **61**:584-592.

140. Davide R, Triantaphyllou A: **Influence of the environment on development and sex differentiation of root-knot nematodes**. *Nematologica* 1967, **13**(1):102-110.
141. Ahmad W: **Studies on the genus *Mesodorylaimus* Andrássy, 1959 from India**. *Nematologica* 1993, **1**(4):417-430.
142. Tauheed U, Ahmad W: **Four new species of the genus *Mesodorylaimus* Andrássy, 1959 (Nematoda: Dorylaimida) from India**. *Zootaxa* 2010, **2642**:19-35.
143. SHAHEEN A, AHMAD W: **Descriptions of four new species of Dorylaimida (Nematoda)**. *Journal of Nematode Morphology and Systematics* 2005, **8**(1):19/29.
144. Botha A, Heyns J: **Freshwater nematodes of the genera *Thornenema* and *Mesodorylaimus* from the Kruger National Park with a diagnostic species compendium for South African species of the genus *Mesodorylaimus* (Nematoda: Dorylaimida)**. *Koedoe-African Protected Area Conservation and Science* 1992, **35**(1):25-42.
145. Abolafia J, Santiago R: **Nematodes of the order Dorylaimida from Andalucía oriental, Spain. The genus *Mesodorylaimus* Andrássy, 1959. IV. Two relatively small sized new species with a compendium of their relatives**. In: *Annales Zoologici (Warszawa): 2000*; 2000: 133-142.
146. Andrássy I: **The genus *Eudorylaimus* Andrássy, 1959 and the present status of its species (Nematoda: Qudsianematidae)**. *Opuscula Zoologica Budapestinensis* 1986, **22**:1-42.
147. Jiang D, Li Q, Liu F, Jiang Y, Liang W: **Vertical distribution of soil nematodes in an age sequence of *Caragana microphylla* plantations in the Horqin Sandy Land, Northeast China**. *Ecol Res* 2007, **22**(1):49-56.
148. Mulvey R: **The Mononchidae: a family of predaceous nematodes: VI. Genus *Mononchus* (Nematoda: Mononchidae)**. *Canadian Journal of Zoology* 1967, **45**(6):915-940.
149. Eisendle U: **Description of *Mononchus sandur* n. sp.(Nematoda: Mononchidae) and remarks on *M. truncatus* Bastian, 1865 and *M. aquaticus* Coetzee, 1968 from a glacial floodplain reach (Grossglockner region, Hohe Tauern, Austria)**. *Nematology* 2008, **10**(6):809-818.
150. Andrassy I: **Three new species of the genus *Mononchus* (Nematoda: Mononchida), and the “real” *Mononchus truncatus* Bastian, 1865**. *Journal of Natural History* 2011, **45**(5-6):303-326.
151. Naseem M, Jairajpuri M, Ahmad I: **Developmental biology of a predatory soil nematode, *Mylonchulus minor***. *Indian Journal of Nematology* 1994, **24**(1):7-15.
152. Mulvey RH: **The Mononchidae: a family of predaceous nematodes: I. Genus *Mylonchulus* (Enoplia: Mononchidae)**. *Canadian Journal of Zoology* 1961, **39**(5):665-696.
153. Fürst von Lieven A, Bärmann V, Sudhaus W: **How can nematodes mate without spicules? Function of the male gonoduct glands in the roundworm *Myolaimus***. *Zoology* 2005, **108**(3):211-216.
154. Baermann E, von Lieven AF, Sudhaus W: **Revision and phylogeny of *Myolaimus* Cobb, 1920 (Secernentea, Nematoda) with the description of four new species**. *Journal of Nematode Morphology and Systematics* 2009, **12**(2):145-168.
155. Anthoine G, Mugniéry D: **Obligatory amphimixis and variation in chromosome number within and among South American populations of *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 (Nematoda: Pratylenchidae)**. *Nematology* 2005, **7**(5):783.

156. Martin J, Lee D: **Changes in the structure of the male reproductive system of *Nematodirus battus* during its rejection from lambs**. *Parasitology* 1980, **81**(03):587-592.
157. Martin J, Lee D: **Observations on the structure of the male reproductive system and spermatogenesis of *Nematodirus battus***. *Parasitology* 1980, **81**(03):579-586.
158. West SA, Gemmil AW, Graham AL, Viney ME, Read AF: **Immune stress and facultative sex in a parasitic nematode**. *J Evol Biol* 2001, **14**:333-337.
159. Phillipson R: **Experiments on the reproduction of *Nippostrongylus brasiliensis* in the rat intestine**. *Parasitology* 1970, **61**(02):317-322.
160. Phillipson R: **Reproduction of *Nippostrongylus brasiliensis* in the rat intestine**. *Parasitology* 1969, **59**(04):961-971.
161. Tarjan A, BAKHUONG N: **A compendium of the family Axonolaimidae,(Nematoda)**. *Cahiers de Biologie Marine* 1988, **29**(3):375-393.
162. Keppner EJ: **Six new species of free-living marine nematodes (Nematoda: Araeolaimida: Enoplida) from two estuaries in Northwest Florida, USA**. *Transactions of the American Microscopical Society* 1988:79-95.
163. Boufahja F, Hedfi A, Essid N, Aïssa P, Mahmoudi E, Beyrem H: **An observational study on changes in biometry and generation time of *Odontophora villoti* (Nematoda, Axonolaimidae) related to petroleum pollution in Bizerte bay, Tunisia**. *Environmental Science and Pollution Research* 2012, **19**(3):646-655.
164. Schulz-Key H: **Observations on the reproductive biology of *Onchocerca volvulus***. *Acta Leidensia* 1989, **59**(1-2):27-44.
165. Schulz-Key H: **[Investigations on the filariidae of the cervidae in Southern Germany. 1. Development of the nodule, finding of the sexes and production of the microfilariae in *Onchocerca flexuosa* (Wedl, 1856), in the red deer (*Cervus elaphus*)(author's transl)]**. *Tropenmedizin und Parasitologie* 1975, **26**(1):60-69.
166. Duke B, Zea-Flores G, Gannon R: **On the reproductive activity of the female *Onchocerca volvulus***. *Tropical Medicine and Parasitology* 1990, **41**(4):387-402.
167. Hildebrandt JC, Eisenbarth A, Renz A, Streit A: **Single worm genotyping demonstrates that *Onchocerca ochengi* females simultaneously produce progeny sired by different males**. *Parasitology Research* 2012, **111**(5):2217-2221.
168. Cupp E, Sauerbrey M, Richards F: **Elimination of human onchocerciasis: history of progress and current feasibility using ivermectin (Mectizan®) monotherapy**. *Acta Tropica* 2011, **120**:S100-S108.
169. Schulz-Key H, Karam M: **Periodic reproduction of *Onchocerca volvulus***. *Parasitology Today* 1986, **2**(10):284-286.
170. Schulz-Key H, Soboslay P: **Reproductive biology and population dynamics of *Onchocerca volvulus* in the vertebrate host**. *Parasite* 1994, **1**:53-55.
171. Coomans A, Verschuren D, Vanderhaeghen R: **The demanian system, traumatic insemination and reproductive strategy in *Oncholaimus oxyuris* Ditlevsen (Nematoda, Oncholaimina)**. *Zoologica Scripta* 1988, **17**(1):15-23.
172. Chilton NB, Huby-Chilton F, Gasser RB, Beveridge I: **The evolutionary origins of nematodes within the order Strongylida are related to predilection sites within hosts**. *Molecular Phylogenetics and Evolution* 2006, **40**(1):118-128.
173. Torres-Barragan A, Suazo A, Buhler WG, Cardoza YJ: **Studies on the entomopathogenicity and bacterial associates of the nematode *Oscheius carolinensis***. *Biological Control* 2011, **59**(2):123-129.

174. Sudhaus W, Hooper DJ: ***Rhabditis* (Oscheius) Guentheri1 Sp. N., an unusual species with reduced posterior ovary, with observations on the Dolichura and Insectivora groups (Nematoda: Rhabditidae)**. *Nematologica, 40* 1994, **1**(4):508-533.
175. Félix M-A: ***Osceius tipulae***. In: *WormBook.* doi/10.1895/wormbook.1.119.1, http://www.wormbook.org: Ed. the *C. elegans* Research Community; 2006.
176. Lancaster M, Hong C: **The identification of females within the subfamily Ostertagiinae Lopez-Neyra 1947**. *Veterinary parasitology* 1990, **35**(1):21-27.
177. Meyvis Y, Houthoofd W, Visser A, Borgonie G, Gevaert K, Vercruysse J, Claerebout E, Geldhof P: **Analysis of the translationally controlled tumour protein in the nematodes *Ostertagia ostertagi* and *Caenorhabditis elegans* suggests a pivotal role in egg production**. *International Journal for Parasitology* 2009, **39**(11):1205-1213.
178. Bergeron E, Measures L, Huot J: **Lungworm (*Otostrongylus circumlitus*) infections in ringed seals (*Phoca hispida*) from eastern Arctic Canada**. *Canadian Journal of Fisheries and Aquatic Sciences* 1997, **54**(10):2443-2448.
179. Sternberg PW, Horvitz HR: **Gonadal cell lineages of the nematode *Panagrellus redivivus* and implications for evolution by the modification of cell lineage**. *Developmental Biology* 1981, **88**(1):147-166.
180. Stock SP, Nadler SA: **Morphological and molecular characterisation of *Panagrellus* spp.(Cephalobina: Panagrolaimidae): taxonomic status and phylogenetic relationships**. *Nematology* 2006, **8**(6):921-938.
181. Stock SP, De Ley P, De Ley I, Mundo-Ocampo M, Baldwin JG, Nadler SA: ***Panagrobelus stammeri* Ruhm, 1956 and *Plectonchus hunti* n. sp.: implications of new morphological observations for characterisation of these genera (Nematoda: Panagrolaimoidea)**. *Nematology* 2002, **4**(3):403-419.
182. Lewis SC, Dyal LA, Hilburn CF, Weitz S, Liau W-S, LaMunyon CW, Denver DR: **Molecular evolution in *Panagrolaimus* nematodes: origins of parthenogenesis, hermaphroditism and the Antarctic species *P. davidi***. *BMC Evolutionary Biology* 2009, **9**(1):15.
183. Pedram M, Niknam G, Vinciguerra M, WeiMin Y, Robbins R: **Description of *Paractinolaimus decraemerae* sp. n. and redescription of *P. parietinus* Eroshenko, 1977 (Dorylaimida: Actinolaimidae) from northwestern Iran**. *International Journal of Nematology* 2010, **20**(2):169-178.
184. Yushin VV: **Spermatid and spermatozoon structure in fresh-water nematode *Paractinolaimus microdentatus* (Nematoda: Dorylaimida: Actinolaimidae)**. *Russian Journal of Nematology* 2010, **18**(2):199-208.
185. Kennedy MJ: ***Filaroides (Parafilaroides) hispidus* n. sp.(Nematoda: Metastrongyloidea) from the lungs of the ringed seal, *Phoca hispida* (Phocidae), from the Beaufort Sea, Canada**. *Canadian Journal of Zoology* 1986, **64**(9):1864-1868.
186. Gosselin J-F, Measures LN: **Redescription of *Filaroides (Parafilaroides) gymnurus* (Railliet, 1899)(Nematoda: Metastrongyloidea), with comments on other species in pinnipeds**. *Canadian Journal of Zoology* 1997, **75**(3):359-370.
187. Dailey MD: **Restoration of *Parafilaroides* (Dougherty, 1946)(Nematoda: Metastrongyloidea) with description of two new species from pinnipeds of eastern central Pacific**. *Journal of Parasitology* 2006, **92**(3):589-594.
188. Dailey MD: **A new species of Parafilaroides (Nematoda: Filaroididae) in three species of fur seals (Carnivora: Otariidae) from the southern hemisphere**. *Journal of Parasitology* 2009, **95**(1):156-159.

189. Goday C, Pimpinelli S: **Cytological analysis of chromosomes in the two species *Parascaris univalens* and *P. equorum***. *Chromosoma* 1986, **94**(1):1-10.
190. Baccetti B, Burrini A, Mazzini M: **The sperm-egg fusion in the nematode *Parascaris equorum***. *International Journal of Invertebrate Reproduction and Development* 1985, **8**(1):15-26.
191. Tomalak M, Welch HE, Galloway TD: **Parasitism of *Parasitorhabditis obtusa* and *P. autographi* (Nematoda: Rhabditidae) in the digestive tract of their bark beetle (Coleoptera: Scolytidae) hosts**. *Journal of Invertebrate Pathology* 1989, **53**(1):57-63.
192. Choo HY, Kaya HK, Shea P, Noffsinger EM: **Ecological study of nematode parasitism in Ips beetles from California and Idaho**. *Journal of Nematology* 1987, **19**(4):495.
193. Carta L, Bauchan G, Hsu C-Y, Yuceer C: **Description of *Parasitorhabditis frontali* n. sp.(Nemata: Rhabditida) from *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae)**. *Journal of Nematology* 2010, **42**(1):46.
194. Meirmans S, Skorping A, Løyning M, Kirkendall L: **On the track of the Red Queen: bark beetles, their nematodes, local climate and geographic parthenogenesis**. *Journal of Evolutionary Biology* 2006, **19**(6):1939-1947.
195. Korenchenko E: **Experimental study of host specificity and species independence of nematodes of the genus *Parasitorhabditis***. In: *Helminths of Insects.* Edited by Sonin M. New Delhi, India: Oxonian Press Pvt. Ltd.; 1990: 110-112.
196. Poinar Jr GO: **Nematodes as facultative parasites of insects**. *Annual Review of Entomology* 1972, **17**(1):103-122.
197. Lindquist W, Hitchcock D: **Studies on infections of a caecal worm, *Paraspidodera uncinata*, in guinea pigs**. *Journal of Parasitology* 1950, **36**(6, Sect, 2, Suppl):37-38.
198. Boag B: **Observations on the population dynamics and vertical distribution of trichodorid nematodes in a Scottish forest nursery**. *Annals of Applied Biology* 1981, **98**(3):463-469.
199. Kumari S: **Description of *Paratrichodorus pachydermus* (Nematoda: Trichodoridae) from the Czech Republic**. *Helminthologia* 2010, **47**(3):196-198.
200. Tarjan AC: **A review of the genus *Paratylenchus* Micoletzky, 1922 (Paratylenchinae: Nematoda) with a description of two new species**. *Annals of the New York Academy of Sciences* 1960, **84**(10):331-332.
201. Geraert E: **The genus *Paratylenchus***. *Nematologica* 1965, **11**(3):301-334.
202. Jenkins W, Taylor D: ***Paratylenchus dianthus*, n.'sp.(Nematoda, Criconematidae), a parasite of carnation**. *Journal of Parasitology* 1858, **37**:26.
203. Raski D: **Revision of the genus *Paratylenchus* Micoletzky, 1922 and descriptions of new species. Part II of three parts**. *Journal of Nematology* 1975, **7**(3):274.
204. Vierstraete TM, Vincx M: **Life strategies in two bacterivorous marine nematodes: preliminary results**. *Marine Ecology* 1996, **17**(1-3):509-518.
205. Derycke S, Remerie T, Backeljau T, Vierstraete A, Vanfleteren J, Vincx M, Moens T: **Phylogeography of the *Rhabditis (Pellioditis)* marina species complex: evidence for long-distance dispersal, and for range expansions and restricted gene flow in the northeast Atlantic**. *Mol Ecol* 2008, **17**(14):3306-3322.
206. Schulte F: **Description of *Rhabditis (Pelodera) pseudoteres* n. sp.(Rhabditidae: Nematoda) with a redescription of its sibling *R.(P.) teres* (Schneider, 1866)**. *Revue de Nématologie* 1989, **12**(4):387-394.

207. Jones T: **Sex, attraction and copulation in *Pelodera teres***. *Nematologica* 1966, **12**(4):518-522.
208. Vik R: **Notes on the life history of *Philonema agubernaculum* Simon et Simon, 1936 (Nematoda)**. *Canadian Journal of Zoology* 1964, **42**(3):511-512.
209. Platzer EG, Adams JR: **The life history of a dracunculoid, *Philonema oncorhynchi*, in Oncorhynchus nerka**. *Canadian Journal of Zoology* 1967, **45**(1):31-43.
210. Zhigileva ON: **Indices of genetic variability of parasites with a different life cycle structure**. *Acta Zoologica Lituanica* 2007, **17**(2):129-138.
211. Bashyrullah A: **The development and maturation of *Philonema* species (Nematoda: Philometridae) in salmonid hosts with different life histories**. Vancouver, Canada: The University of British Columbia; 1966.
212. Lahl V, Halama C, Schierenberg E: **Comparative and experimental embryogenesis of Plectidae (Nematoda)**. *Development Genes and Evolution* 2003, **213**(1):18-27.
213. Bostrom S: **Populations of *Plectus acuminatus* Bastian. 1865 and *Panagrolaimus magnivulvatus* n. sp.(Nematoda) from nunatakks in Dronning Maud Land, East Antarctica**. *Fundamental and Applied Nematology* 1995, **18**(1):25-34.
214. Kammenga JE, Van Koert PH, Riksen JA, Korthals GW, Bakker J: **A toxicity test in artificial soil based on the life-history strategy of the nematode *Plectus acuminatus***. *Environmental Toxicology and Chemistry* 1996, **15**(5):722-727.
215. Holovachov O: **Morphology, phylogeny and evolution of the superfamily Plectoidea Örley, 1880 (Nematoda: Plectida)**. *Annales Zoologici* 2004, **54**:631-672.
216. Hong RL, Villwock A, Sommer RJ: **Cultivation of the rhabditid *Poikilolaimus oxycercus* as a laboratory nematode for genetic analyses**. *Journal of Experimental Zoology Part A: Comparative Experimental Biology* 2005, **303**(9):742-760.
217. Yushin VV, Coomans A, Malakhov VV: **Ultrastructure of spermatogenesis in the free-living marine nematode *Pontonema vulgare* (Enoplida, Oncholaimidae)**. *Canadian Journal of Zoology* 2002, **80**(8):1371-1382.
218. Belogurov O, Belogurova L: **Morphology and systematics of free-living Oncholaimidae (Nematoda: Enoplida: Oncholaimina)**. *Asian Marine Biology* 1989, **6**:31-58.
219. Frantová D: **On the morphology and surface ultrastructure of some parasitic nematodes (Nematoda) of birds (Aves)**. *Acta Societatis Zoologicae Bohemicae* 2002, **66**:85-97.
220. Fagerholm H-P, Overstreet RM: **Ascaridoid nematodes: *Contracaecum, Porrocaecum,* and *Baylisascaris***. In: *Parasitic Diseases of Wild Birds.* Edited by Atkinson C, Thomas N, Hunter D. Hoboken, NJ: John Wiley & Sons, Inc.; 2008: 413-433.
221. Vovlas N, Inserra R: **The systematic position of *Praytlenchoides ritteri* Sher with observations on its embryogenic development**. *Nematologia Mediterranea* 1978, **6**:49-56.
222. Sher S: **Revision of the genus *Pratylenchoides* Winslow, 1958 (Nematoda: Tylenchoidea)**. *Proceedings of the Helminthological Society of Washington* 1970, **37**(2):154-166.
223. Baldwin JG, Luc M, Bell A: **Contribution to the study of the genus *Pratylenchoides* Winslow (Nematoda: Tylenchida)**. *Revue de Nématologie* 1983, **6**(1):111-125.
224. De Waele D, Elsen A: **Migratory endoparasites: *Pratylenchus* and *Radopholus* species**. In: *Plant Resistance to Parasitic Nematodes.* Edited by Starr J, Cook R, Bridge J. New York, NY: CAB International; 2002: 175-206.

225. Corbett D: **Three new species of *Pratylenchus* with a redescription of *P. andinus* Lordello, Zamith Boock, 1961 (Nematoda: Pratylenchidae)**. *Nematologica* 1983, **29**(4):390-403.
226. Nico A, Vovlas N, Troccoli A, Castillo P: **Reproduction of the banana root-lesion nematode, *Pratylenchus goodeyi*, in monoxenic cultures**. *Nematologia Mediterranea* 1999, **27**:187-192.
227. Mulvey RH: **The Mononchidae: a family of predaceous nematodes. VII. Genus *Prionchulus* (Nematoda: Mononchidae)**. *Canadian Journal of Zoology* 1967, **43**:941-953.
228. Geraert E, Acek Z, Sudhaus W: **The facultative insect parasite *Pristonchus lheritieri* (syn. *P. Pseudolheritieri*)(Nematoda: Diplogasterida)**. *Nematologica* 1989, **35**(2):133-141.
229. Herrmann M, Mayer WE, Sommer RJ: **Nematodes of the genus *Pristionchus* are closely associated with scarab beetles and the Colorado potato beetle in Western Europe**. *Zoology* 2006, **109**(2):96-108.
230. Mayer WE, Herrmann M, Sommer RJ: **Phylogeny of the nematode genus *Pristionchus* and implications for biodiversity, biogeography and the evolution of hermaphroditism**. *BMC Evolutionary Biology* 2007, **7**(1):104.
231. Dieterich C, Clifton SW, Schuster LN, Chinwalla A, Delehaunty K, Dinkelacker I, Fulton L, Fulton R, Godfrey J, Minx P: **The *Pristionchus pacificus* genome provides a unique perspective on nematode lifestyle and parasitism**. *Nature Genet* 2008, **40**(10):1193-1198.
232. Paggi L, Nascetti G, Cianchi R, Orecchia P, Mattiucci S, D'amelio S, Berland B, Brattey J, Smith J, Bullini L: **Genetic evidence for three species within *Pseudoterranova decipiens* (Nematoda, Ascaridida, Ascaridoidea) in the North Atlantic and Norwegian and Barents seas**. *International Journal for Parasitology* 1991, **21**(2):195-212.
233. Nadler SA, D'Amelio S, Dailey MD, Paggi L, Siu S, Sakanari JA: **Molecular phylogenetics and diagnosis of *Anisakis, Pseudoterranova,* and *Contracaecum* from Northern Pacific marine mammals**. *Journal of Parasitology* 2005, **91**(6):1413-1429.
234. Goodey T: **On *Pungentus thornei* n. sp., a new dorylaimid nematode from soil**. *Journal of Helminthology* 1942, **20**(1-2):6-9.
235. Loos C: **Studies on the life-history and habits of the burrowing nematode, *Radopliolus similis*, the cause of black-head disease of banana**. *Proceedings of the Helminthological Society of Washington* 1962, **29**(1):43-52.
236. LaMunyon CW, Samuel W: **Evolution of sperm size in nematodes: sperm competition favours larger sperm**. *Proceedings of the Royal Society of London Series B: Biological Sciences* 1999, **266**(1416):263-267.
237. Kiontke K, Sudhaus W: **Ecology of *Caenorhabditis* species**. In: *WormBook.* doi/10.1895/wormbook.1.37.1, http://www.wormbook.org: Ed. the *C. elegans* Research Community; 2006.
238. Park HW, Kim YO, Ha J-S, Youn SH, Kim HH, Bilgrami AL, Shin CS: **Effects of associated bacteria on the pathogenicity and reproduction of the insect-parasitic nematode *Rhabditis blumi* (Nematoda: Rhabditida)**. *Canadian Journal of Microbiology* 2011, **57**(9):750-758.
239. Matandala M, Mugera G, Ngatia T: **Prevalence of bovine (nematodes) otitis in Kenya**. *Kenya Veterinarian* 2007, **25**(1):32-35.
240. Poinar G: ***Rhabditis myriophila* sp. n.(Rhabditidae: Rhabditida), associated with the millipede *Oxidis gracilis* (Polydesmida: Diplopoda)**. *Proceedings of the Helminthological Society of Washington* 1986, **53**(2):232-236.

241. Willems M, Houthoofd W, Claeys M, Couvreur M, Van Driessche R, Adriaens D, Jacobsen K, Borgonie G: **Unusual intestinal lamellae in the nematode *Rhabditophanes* sp. KR3021 (Nematoda: Alloinematidae)**. *Journal of Morphology* 2005, **264**(2):223-232.
242. Houthoofd W, Willems M, Jacobsen K, Coomans A, Borgonie G: **The embryonic cell lineage of the nematode *Rhabditophanes* sp**. *International Journal of Developmental Biology* 2008, **52**:963-967.
243. Boag B: **Observations on the population dynamics, life cycle and ecology of the plant parasitic nematode *Rotylenchus robustus***. *Annals of Applied Biology* 1982, **100**(1):157-165.
244. Vovlas N, Subbotin SA, Troccoli A, Liébanas G, Castillo P: **Molecular phylogeny of the genus *Rotylenchus* (Nematoda, Tylenchida) and description of a new species**. *Zoologica Scripta* 2008, **37**(5):521-537.
245. Moura R, Teixeira L: **Morphological aspects of *Scutellonema bradys* (Steiner & Lehew, 1933) Andrassy, 1958 (Nematoda: Hoplolaiminae)**. *Fitopatologia Brasileira* 1980, **5**(3):359-367.
246. Overhoff A, Freckman DW, Virginia RA: **Life cycle of the microbivorous Antarctic Dry Valley nematode *Scottnema lindsayae* (Timm 1971)**. *Polar Biology* 1993, **13**(3):151-156.
247. Andrássy I: **A dozen new nematode species from Hungary**. *Opuscula Zoologica Budapest* 1985, **19**:3-39.
248. Mohanty M, Sahoo P, Satapathy A, Ravindran B: ***Setaria digitata* infections in cattle: parasite load, microfilaraemia status and relationship to immune response**. *Journal of Helminthology* 2000, **74**(04):343-347.
249. Liang-Sheng Y: **A revision of the nematode genus *Setaria* Viborg, 1795, its hostparasite relationship, speciation and evolution**. *Journal of Helminthology* 1959, **33**:1-98.
250. Bezdek H: **Studies on the nematode *Soboliphyme baturini* Petrow, 1930**. *Transactions of the American Microscopical Society* 1942, **61**(4):398-403.
251. Zarnke RL, Whitman JS, Flynn RW, Ver Hoef JM: **Prevalence of *Soboliphyme baturini* in marten (*Martes americana*) populations from three regions of Alaska, 1990-1998**. *Journal of Wildlife Diseases* 2004, **40**(3):452-455.
252. Koehler AV, Hoberg EP, Dokuchaev NE, Tranbenkova NA, Whitman JS, Nagorsen DW, Cook JA: **Phylogeography of a Holarctic nematode, *Soboliphyme baturini,* among mustelids: climate change, episodic colonization, and diversification in a complex host–parasite system**. *Biological Journal of the Linnean Society* 2009, **96**(3):651-663.
253. Swartz L: **A new natural definitive host, and morphometry of *Soboliphyme baturini* Petrow, 1930 in interior Alaska**. *Canadian Journal of Zoology* 1968, **46**(4):691-693.
254. Poinar G: **Description and taxonomic position of DD-136 nematode (Steinernematidae Rhabditoidea) and its relationship to *Neoaplectana carpocapsae* Weiser**. *Proceedings of the Helminthological Society of Washington* 1967, **34**(2):199-&.
255. Viney M, Matthews B, Walliker D: **Mating in the nematode parasite *Strongyloides ratti*: proof of genetic exchange**. *Proceedings of the Royal Society of London Series B: Biological Sciences* 1993, **254**(1341):213-219.
256. Streit A: **Reproduction in *Strongyloides* (Nematoda): a life between sex and parthenogenesis**. *Parasitology* 2008, **135**(03):285-294.
257. McCraw B, Slocombe J: ***Strongylus equinus*: development and pathological effects in the equine host**. *Canadian Journal of Comparative Medicine* 1985, **49**(4):372.

258. Ogbourne CP: **Studies on the epidemiology of *Strongylus vulgaris* infection of the horse**. *International Journal for Parasitology* 1975, **5**(4):423-426.
259. Gibbons LM: **An SEM study of the cephalic region, buccal cavity and male tail of the species of the genus *Strongylus* Müller, 1780 (Nematoda, Strongyloidea)**. *Journal of Helminthology* 1984, **58**(02):149-158.
260. Mercer C: **New record of the grass root-gall nematode *Subanguina radicicola* (Nematoda: Anguininae)**. *New Zealand journal of Experimental Agriculture* 1986, **14**(2):215-217.
261. Mitkowski N, Jackson N: ***Subanguina radicicola*, the root-gall nematode infecting *Poa annua* in New Brunswick, Canada**. *Plant Disease* 2003, **87**(10):1263-1263.
262. Campbell JW: **The gapeworm (*Syngamus*) in wild birds**. *The Journal of Animal Ecology* 1935, **4**(2):208-215.
263. Wehr EE: **Observations on the development of the poultry gapeworm *Syngamus trachea***. *Transactions of the American Microscopical Society* 1937:72-78.
264. Fernando M, Stockdale P, Remmier O: **The route of migration, development, and pathogenesis of *Syngamus trachea* (Montagu, 1811) Chapin, 1925, in pheasant**. *Journal of Parasitology* 1971, **57**:107-116.
265. de Man JG: **Sur quelques nématodes libres de la Mer du Nord, nouveaux ou peu connus**. *Mémoires de la Société zoologique de France* 1888, **1**:1-51.
266. Lima R, Lins L, Da Silva M, Esteves A: **Four new species of *Syringolaimus* De Man, 1888 (Nematoda: Ironidae) from the Southeast Atlantic (Brazil), with redefinition of valid species and the proposal of a new key**. *Zootaxa* 2009, **2096**:119-136.
267. Hopper BE: **Foliicolous marine nematodes on turtle grass, *Thalassia testudinum* Konig, in Biscayne Bay, Florida**. *Bulletin of Marine Science* 1967, **17**(2):471-517.
268. Anderson R: **Comparative morphology and descriptions of three new species of *Teratocephalus* from Canada**. *Canadian Journal of Zoology* 1969, **47**(5):829-840.
269. Boström S, Holovachov O, Susulovsky A: **Study of Teratocephalidae (Nematoda) from the Ukraine. Description of a population of *Teratocephalus* de Man, 1876 with a compendium on species from the" *lirellus*-group"**. *Russian Journal of Nematology* 2000, **8**(2):139-145.
270. Kanzaki N, Abe F, Giblin-Davis RM, Kiontke K, Fitch DH, Hata K, Sone K: ***Teratorhabditis synpapillata* Sudhaus, 1985 (Rhabditida: Rhabditidae) is an associate of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)**. *Nematology* 2008, **10**(2):207-218.
271. Sudhaus W: **Revision der Untergattung Teratorhabditis von *Rhabditis* (Nematoda) und Beschreibung einer neuen Art**. *Zoologische Jahrbücher Abteilung für Systematik, Ökologie und Geographie der Tiere* 1985, **112**(2):207-224.
272. Sprent J: **Ascaridoid nematodes of amphibians and reptiles: *Terranova***. *Journal of Helminthology* 1979, **53**(3):265-282.
273. Traunspurger W: **Distribution, seasonal occurrence and vertical pattern of *Tobrilus gracilis* (Bastian, 1865) and *T. medius* (Schneider, 1916)**. *Nematologica* 1997, **43**(1):59-81.
274. Traunspurger W: **The biology and ecology of lotic nematodes**. *Freshwater Biology* 2000, **44**(1):29-45.
275. Schiemer F, Duncan A: **The oxygen consumption of a freshwater benthic nematode, *Tobrilus gracilis* (Bastian)**. *Oecologia* 1974, **15**(2):121-126.

276. Mutafova T: **Meiosis and some aspects of cytological mechanisms of chromosomal sex determination in nematode species**. *International Journal for Parasitology* 1995, **25**(4):453-462.
277. Sprent J: **The life history and development of *Toxascaris leonina* (von Linstow 1902) in the dog and cat**. *Parasitology* 1959, **49**(3-4):330-371.
278. Gagliardo L, McVay C, Appleton J: **Molting, ecdysis, and reproduction of *Trichinella spiralis* are supported in vitro by intestinal epithelial cells**. *Infection and Immunity* 2002, **70**(4):1853-1859.
279. Kumari S, Decraemer W: **First report of *Trichodorus primitivus, T. sparsus and T. viruliferus* (Nematoda: Trichodoridae) from the Czech Republic**. *Helminthologia* 2011, **48**(3):195-199.
280. Špakulová M, Králová I, Cutillas C: **Studies on the karyotype and gametogenesis in *Trichuris muris***. *Journal of Helminthology* 1994, **68**(01):67-72.
281. Fitch D: **Worm Systematic Resource Network**. In*.* http://www.nyu.edu/projects/fitch/WSRN/; 1998.
282. Siddiqi M: **Nematodes of tropical rainforests: 2. Five new genera and eight new species of cephalobs**. *Afro-Asian Journal of Nematology* 1993, **3**(2):212-225.
283. Schuurmans Stekhoven JJ: **The free living nematodes of the Mediterranean II. The Camargue**. *Zoologische Mededeelingen* 1942, **23**:217-228.
284. Tchesunov AV, Mokievsky VO, Thanh NV: **Three new free-living nematode species (Nematoda, Enoplida) from mangrove habitats of Nha Trang, Central Vietnam**. *Russian Journal of Nematology* 2010, **18**(2):155-173.
285. Khera S: **Studies on the genus *Tripyla* Bastian (Nematoda: Tripylidae)**. In: *Proceedings of the Zoological Society: 1970*: Calcutta; 1970: 195-200.
286. Kisiel M, Zuckerman B: **Studies on aging of *Turbatrix aceti***. *Nematologica* 1974, **20**(3):277-282.
287. Vinciguerra M: **New and known species of *Tylencholaimus* de Man, 1876 (Dorylaimida, Nematoda) from Italian beech forests with a key to the species**. *Nematologia Mediterranea* 1986, **14**:107-116.
288. Ahmad W, Park B-Y, Lee J-K, Choi D-R: **Some new and known species of Tylencholaimoidea (Nematoda: Dorylaimida) from Korea**. *Journal of Natural History* 2009, **43**(37-38):2329-2356.
289. Okada H, Harada H, Tsukiboshi T, Araki M: **Characteristics of *Tylencholaimus parvus* (Nematoda: Dorylaimida) as a fungivorus nematode**. *Nematology* 2005, **7**(6):843-850.
290. Anderson R: **Description of the male of *Tylenchorhynchus maximus* Allen, 1955 (Nematoda: Tylenchorhynchinae)**. *Canadian Journal of Zoology* 1977, **55**(11):1921-1923.
291. Allen MW: **A review of the nematode genus *Tylenchorhynchus***. *University of California Publications in Zoology* 1955, **61**:129-166.
292. Bridge J: **Hatching of *Tylenchorhynchus maximus* and *Merlinius icarus***. *Journal of Nematology* 1974, **6**(2):101.
293. Van Gundy S: **The life history of the citrus nematode *Tylenchulus semipenetrans* Cobb** *Nematologica* 1958, **3**(4):283-294.
294. De Ley P, Coomans A: **Terrestrial nematodes from the Galapagos Archipelago. 7. Description of *Tylocephalus nimius* sp. n. and new data on the morphology, development and behaviour of *T. auriculatus* (Bütschli, 1873) Anderson, 1966 (Leptolaimina: Plectidae)**. *Fundamental and Applied Nematology* 1997, **20**(3):213-228.

295. Brzeski M: **Synopsis of *Tylolaimophorus* De Man, 1880 (Nematoda: Diphtherophoridae)**. *Nematologica, 40* 1994, **1**(4):313-327.
296. Hopper BE, Meyers SP: **Aspects of the life cycle of marine nematodes**. *Helgoländer wissenschaftliche Meeresuntersuchungen* 1966, **13**(4):444-449.
297. Smol N, Sharma J: **Two new and three redescribed species of *Viscosia* (Nematoda, Oncholaimidae)**. *Hydrobiologia* 1984, **114**(2):123-147.
298. Holovachov O, Boström S, de Ley IT, De Ley P, Coomans A: **Morphology and systematics of the genera *Wilsonema* Cobb, 1913, Ereptonema Anderson, 1966 and Neotylocephalus Ali, Farooqui & Tejpal, 1969 (Leptolaimina: Wilsonematinae)**. *Journal of Nematode Morphology and Systematics* 2002, **5**(1):73-106.
299. Miller MJ: **Observations on spermatogenesis in *Onchocerca volvulus* and *Wuchereria bancrofti***. *Canadian Journal of Zoology* 1966, **44**(6):1003-1006.
300. Araujo AC, Figueredo-Silva J, Souto-Padrón T, Dreyer G, Norões J, De Souza W: **Scanning electron microscopy of adult *Wuchereria bancrofti* (Nematoda: Filarioidea)**. *The Journal of Parasitology* 1995:468-474.
301. De Kraker ME, Stolk WA, Van Oortmarssen GJ, Habbema JDF: **Model-based analysis of trial data: microfilaria and worm productivity loss after diethylcarbamazine–albendazole or ivermectin–albendazole combination therapy against *Wuchereria bancrofti***. *Tropical Medicine & International Health* 2006, **11**(5):718-728.
302. Vandekerckhove T, Willems A, Gillis M, Coomans A: **Occurrence of novel verrucomicrobial species, endosymbiotic and associated with parthenogenesis in *Xiphinema americanum*-group species (Nematoda, Longidoridae)**. *International Journal of Systematic and Evolutionary Microbiology* 2000, **50**(6):2197-2205.
303. Allen M, Noffsinger E: **A review of the genus *Zeldia* Thome, 1937 (Nematoda: Cephalobidae) with descriptions of seven new species**. *Proceedings of the Helmithological Society of Washington* 1972, **39**:206-223.
304. Karakaş M: **Life cycle and mating behavior of *Zygotylenchus guevarai* (Nematoda: Pratylenchidae) on excised *Petroselinum crispum* roots**. *Pakistan Journal of Biological Sciences* 2007, **10**(22).
305. Verdejo S, Pinochet J: **Reproduction of the nematode *Zygotylenchus guevarai* (Nemata: Pratylenchidae) in monoxenic cultures**. *Revue de Nématologie* 1991, **14**(2):291-297.
306. Meldal B, Debenham N, De Ley P, De Ley I, Vanfleteren J, Vierstraete A, Bert W, Borgonie G, Moens T, Tyler P *et al*: **An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa**. *Mol Phyl Evol* 2007, **42**:622-636.
307. Hurst LD, Peck JR: **Recent advances in understanding of the evolution and maintenance of sex**. *Trends Ecol Evol* 1996, **11**(2):46-52.
308. Agrawal AF, Lively CM: **Parasites and the evolution of self-fertilization**. *Evolution* 2001, **55**(5):869-879.
309. Barrière A, Félix MA: **High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations**. *Curr Biol* 2005, **15**(13):1176-1184.
310. Maddison WP, FitzJohn RG: **The unsolved challenge to phylogenetic correlation tests for categorical characters**. *Syst Biol* 2014, ***in press***.

311. Blaxter M, De Ley P, Garey J, Liu L, Scheldeman P, Vierstraete A, Vanfleteren J, Mackey L, Dorris M, Frisse L *et al*: **A molecular evolutionary framework for the phylum Nematoda**. *Nature* 1998, **392**:71-75.
312. Dorris M, De Ley P, Blaxter ML: **Molecular analysis of nematode diversity and the evolution of parasitism**. *Parasitol Today* 1999, **15**(5):188-193.
313. De Ley P: **A quick tour of nematode diversity and the backbone of nematode phylogeny**. In: *WormBook.* doi/10.1895/wormbook.1.41.1, http://www.wormbook.org: Ed. the *C. elegans* Research Community; 2006.
314. van Megen H, van den Elsen S, Holterman M, Karssen G, Mooyman P, Bongers T, Holovachov O, Bakker J, Helder J: **A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences**. *Nematology* 2009, **11**(6):927-950.
315. Kiontke K, Felix MA, Ailion M, Rockman M, Braendle C, Penigault J-B, Fitch D: **A phylogeny and molecular barcodes for *Caenorhabditis*, with numerous new species from rotting fruits**. *BMC Evol Biol* 2011, **11**(1):339.
316. Kiontke K, Fitch D: **The phylogenetic relationships of *Caenorhabditis* and other rhabditids**. *WormBook, ed The C elegans Research Community* 2005, **(August 11 2005)**:WormBook, doi/10.1895/wormbook.1891.1811.1891, http://www.wormbook.org.
317. Kiontke K, Gavin NP, Raynes Y, Roehrig C, Piano F, Fitch DH: ***Caenorhabditis* phylogeny predicts convergence of hermaphroditism and extensive intron loss**. *Proc Nat Acad Sci USA* 2004, **101**(24):9003-9008.
318. Cutter AD, Wasmuth JD, Washington NL: **Patterns of molecular evolution in *Caenorhabditis* preclude ancient origins of selfing**. *Genetics* 2008, **178**(4):2093-2104.
319. Denver DR, Clark KA, Raboin MJ: **Reproductive mode evolution in nematodes: insights from molecular phylogenies and recently discovered species**. *Mol Phyl Evol* 2011, **61**(2):584-592.
320. Bollback JP: **SIMMAP: Stochastic character mapping of discrete traits on phylogenies**. *BMC Bioinformatics* 2006, **7**:88.
321. Goldberg E, Igic B: **On phylogenetic tests of irreversible evolution**. *Evolution* 2008, **62**(11):2727-2741.
322. Igic B, Bohs L, Kohn JR: **Ancient polymorphism reveals unidirectional breeding system shifts**. *Proc Nat Acad Sci USA* 2006, **103**(5):1359-1363.
323. Freckleton RP, Harvey PH, Pagel M: **Phylogenetic analysis and comparative data: a test and review of evidence**. *Am Nat* 2002, **160**(6):712-726.
324. Ackerly DD: **Taxon sampling, correlated evolution, and independent contrasts**. *Evolution* 2000, **54**(5):1480-1492.
325. Bell G: **The Masterpiece of Nature: the Evolution and Genetics of Sexuality**. Berkeley, CA: University of California Press; 1982.
326. Igic B, Kohn JR: **The distribution of plant mating systems: study bias against obligately outcrossing species**. *Evolution* 2006, **60**(5):1098-1103.
327. R Core Team: **R: a language and environment for statistical computing**. *R Foundation for Statistical Computing* 2013, **Vienna, Austria**:http://www.R-project.org.
328. Revell LJ: **phytools: an R package for phylogenetic comparative biology (and other things)**. *Methods in Ecology and Evolution* 2012, **3**(2):217-223.

329. Huelsenbeck JP, Nielsen R, Bollback JP: **Stochastic mapping of morphological characters**. *Syst Biol* 2003, **52**(2):131-158.
330. Nielsen R: **Mapping mutations on phylogenies**. *Syst Biol* 2002, **51**(5):729-739.
331. Pagel M: **Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters**. *Proc Roy Soc B* 1994, **255**(1342):37-45.
332. Beaulieu JM, O'Meara BC, Donoghue MJ: **Identifying Hidden Rate Changes in the Evolution of a Binary Morphological Character: The Evolution of Plant Habit in Campanulid Angiosperms**. *Systematic Biology* 2013, **62**(5):725-737.
333. Meredith RW, Pires MN, Reznick DN, Springer MS: **Molecular phylogenetic relationships and the coevolution of placentotrophy and superfetation in *Poecilia* (Poeciliidae: Cyprinodontiformes)**. *Mol Phyl Evol* 2011, **59**(1):148-157.
334. Soza V, Brunet J, Liston A, Smith P, Di Stilio V: **Phylogenetic insights into the correlates of dioecy in meadow-rues (*Thalictrum,* Ranunculaceae)**. *Mol Phyl Evol* 2012, **63**:180-192.
335. Blanco-Pastor J, Vargas P: **Autecological traits determined two evolutionary strategies in Mediterranean plants during the Quaternary: low differentiation and range expansion versus geographical speciation in *Linaria***. *Mol Ecol* 2013, **22**(22):5651-5668.
336. Jones CS, Bakker FT, Schlichting CD, Nicotra AB: **Leaf shape evolution in the south African genus *Pelargonium* l’Hér.(Geraniaceae)**. *Evolution* 2009, **63**(2):479-497.