



## Research

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# The trophic vacuum and the evolution of complex life cycles in trophically transmitted helminths

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Parasitic worms (helminths) frequently have complex life cycles in which they are transmitted trophically between two or more successive hosts. Sexual reproduction often takes place in high trophic-level (TL) vertebrates, where parasites can grow to large sizes with high fecundity. Direct infection of high TL hosts, while advantageous, may be unachievable for parasites constrained to transmit trophically, because helminth propagules are unlikely to be ingested by large predators. Lack of niche overlap between propagule and definitive host (the trophic transmission vacuum) may explain the origin and/or maintenance of intermediate hosts, which overcome this transmission barrier. We show that nematodes infecting high TL definitive hosts tend to have more successive hosts in their life cycles. This relationship was modest, though, driven mainly by the minimum TL of hosts, suggesting that the shortest trophic chains leading to a host define the boundaries of the transmission vacuum. We also show that alternative modes of transmission, like host penetration, allow nematodes to reach high TLs without intermediate hosts. We suggest that widespread omnivory as well as parasite adaptations to increase transmission probably reduce, but do not eliminate, the barriers to the transmission of helminths through the food web.

## 1. Introduction

Helminths include members of very different and only distantly related metazoans that are united by their parasitic mode of life and the complexity of their life cycles. Complex life cycles (i.e. cycles with successive transmission through a number of hosts) are common in nematodes and the rule in cestodes, trematodes and acanthocephalans [1–4]. Transmission between hosts is frequently trophic; one host is eaten by the next host. The transmission of parasites through the food web can be quite tortuous, e.g. the nematode *Anisakis simplex* can infect from three to five hosts before reproducing [5]. The requirement for several hosts is puzzling at first glance, because the probability of completing the life cycle presumably decreases as it gets longer [6]. Moreover, parasites with direct, one-host cycles do not have to deal with multiple host physiologies and immune systems. While such ‘costs of complex cycles’ need not always apply [7], they beg the question as to whether there are also well-defined benefits associated with trophic transmission and longer life cycles.

Helminths frequently reproduce in high trophic-level (TL) vertebrates [1] that tend to be large and long-lived [8–10]. Given the higher energy intake and longevity of such hosts, helminths likely achieve high growth rates in them with relatively low mortality rates, conditions favouring the evolution of larger reproductive sizes and fecundities [11]. Of course, some factors may complicate these expectations, e.g. high TL vertebrates may have a more sophisticated and effective immune response. Nonetheless, parasites usually undergo extensive growth in their definitive hosts [12,13], particularly when hosts are large [14–17]. Direct infection of large vertebrates, though seemingly

advantageous, may be difficult for helminths that are transmitted trophically, simply because their propagules are small and unlikely to fall in the normal size spectrum of a large predator's diet [18]. We refer to this problem as the trophic transmission vacuum; because propagules are trophically unavailable to a definitive host, they have very low, perhaps negligible, transmission probability across non-adjacent TLs by trophic ingestion. The incorporation of intermediate hosts into the life cycle may be a way to escape the trophic vacuum and achieve transmission to larger, more favourable hosts for reproduction, higher up the food chain [19–22].

However, for parasites constrained to transmit trophically, the extent to which a trophic transmission vacuum exists between free-living propagules and high TL definitive hosts is unclear. Some predators (e.g. lions) consume almost exclusively from the TL immediately below them (grazing ungulates), which clearly imposes a constraint on parasites infecting via an oral route. With a 'perfect' trophic vacuum between TLs, to attain residency and reproduce in a top predator (say at TL 4) would require a helminth to exploit intermediate hosts in each TL (2 and 3) below that predator. But many animals consume food from several TLs [23], e.g. bears consume anything from vegetation to other (often large) vertebrates. Taken to the extreme, if species consumed freely from all lower TLs (i.e. widespread omnivory), there would be no trophic vacuum. Definitive hosts at high TLs could readily be infected by even small parasite propagules, so intermediate hosts would be unnecessary and probably even costly, assuming that generalism, the ability to infect multiple host species, carries a cost. In such circumstances, we might expect the evolution of direct (one-host) cycles, with definitive hosts at high TLs as the sole hosts. Regardless of the levels of omnivory in food webs, parasites have adaptations to increase the probability of seemingly unlikely trophic transmission events. The manipulation of host behaviour to increase predation susceptibility is one well-documented example [24] and egg structures that increase the chances of accidental consumption is another [25].

The trophic vacuum may be critical in the evolutionary origin and maintenance of complex life cycles in trophically transmitted helminths [26]. There are two ways in which a life cycle can be extended by adding an extra host: (i) in downward incorporation, a new intermediate host is added below the definitive host and (ii) in upward incorporation, a predator of the original host becomes the new definitive host, and the original host becomes an intermediate host (e.g. [19,26,27]). For downward incorporation, a trophic vacuum that arises below the definitive host (for evolutionary or ecological reasons) can favour the incorporation and subsequent maintenance of an intermediate host [26]. By contrast, upward incorporation does not require a trophic vacuum; there is no trophic vacuum between the original host and its predator one TL above. However, since there are likely to be costs associated with having multiple hosts, for both upward and downward incorporation, we envisage that the trophic vacuum may be an important selective force (along with phylogenetic constraints, e.g. [28]) maintaining intermediate hosts in complex cycles [26].

Ultimately, a well-defined trophic transmission vacuum is expected to occur when predation across non-adjacent TLs has low probability. We investigated how strongly the number of successive hosts in a trophically transmitted

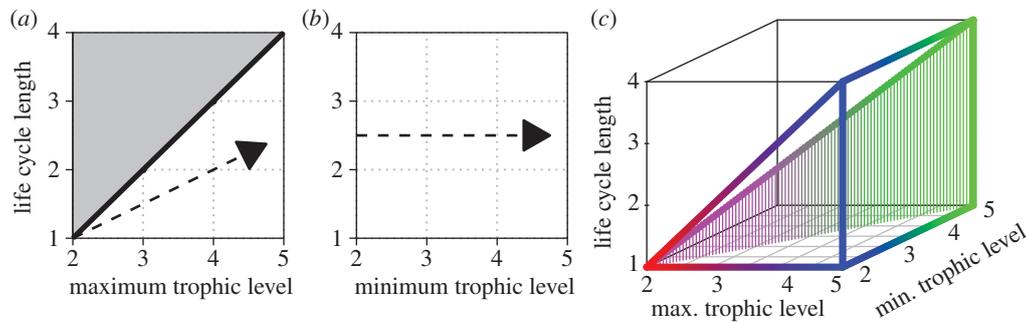
parasite's life cycle correlates with the TL of the definitive host, as this may indicate the extent to which food webs create a trophic vacuum. On the one hand, trophically transmitted parasites with complex life cycles rely on a series of consumption events in which successive hosts are usually eaten by larger hosts [9,18,29,30], so a positive correlation is expected. However, omnivory is widespread [23], so there is no *a priori* reason why high TL hosts should house only helminths with complex life cycles; without a trophic vacuum, direct trophic transmission to top predators is possible. A perfect trophic vacuum (i.e. parasites must go through each TL leading to the definitive host) should result in an exact correspondence between life cycle length (LCL) and the TL of the definitive host (regression slope 1). A perfect lack of any barrier should result in no correlation (regression slope 0). We use nematode parasites of vertebrates, because, relative to other helminth groups, nematodes vary considerably in life cycle complexity [1]. Nematodes commonly infect one, two or three hosts, and less often more, before completing the life cycle. Our analysis suggests that the trophic vacuum may be weaker than expected. Some nematodes transmit by means other than trophic ingestion; for example, many penetrate their hosts directly and could thus avoid any trophic vacuum. While our study relates primarily to parasites constrained to transmit by ingestion, we also explore whether two alternative nematode transmission strategies (penetration and vector transmission) allow parasites to reach high TL definitive hosts with fewer hosts in the life cycle, compared with trophic transmission.

## 2. Material and methods

### (a) Data collection

We collected data on LCL and definitive host TL for parasitic nematodes. Anderson [31] summarized the life cycles of nematodes infecting vertebrates, and this was our primary source for data on LCL. We also added information for several species whose life cycles have been more recently elucidated [32–37]. A number of species in the dataset have flexible life cycles in that some hosts are used only facultatively. For example, the nematode *Camallanus cotti* normally has a two-host life cycle in which it infects copepods as first host and fish as second host. However, it can also infect fish directly, so the copepod first host is actually facultative [38]. To accommodate such cases (16.3% of the 392 trophically transmitted species in the dataset), we ran all analyses twice, once with facultative species assumed to have the longest possible life cycle and once assuming the shortest life cycle (in the case of *C. cotti*, two hosts or one host). Throughout, when we refer to long or short LCL, we are referring to the number of hosts in a parasite life cycle, not the time required to complete the cycle.

Species-level estimates of TL are usually made from food webs or quantitative diet studies, which are limited in their taxonomic scope. Precise TL information is not available on a broad phylogenetic scale, an exception being FishBase, which provides estimates of weighted mean TL for fishes [39]. Thus, for some nematode species in the dataset we had specific estimates of the TL of the definitive hosts, but for many species we did not. To fill these gaps in the data, we defined discrete TLs as follows. We assumed basal producer taxa (e.g. plants, phytoplankton and detritus) to have TL = 1. Higher levels were: TL = 2 herbivores (primary consumers), TL = 3 primary carnivores eating herbivores (secondary consumers), TL = 4 carnivores eating other carnivores (tertiary consumers) and TL = 5, apex predators.



**Figure 1.** Complex life cycle parasites cannot sequentially infect more hosts than the maximum trophic chain length leading to their definitive host. Given that animal-infecting nematodes begin their life cycle in a host at a TL  $\geq 2$ , this constrains the ‘samplable’ phenotype space to  $LCL \leq \max TL - 1$ , as shown in (a). As a consequence, a positive correlation between LCL and max TL is expected, even when species are randomly distributed within this space. (b) Min TL, by contrast, need not covary with LCL, because even hosts with a high min TL could acquire trophically transmitted parasites through accidental ingestion. (c) The observable combinations of nematode LCL, min TL and max TL in three dimensions. A structured permutation (see text) tested whether observed associations between LCL and TL departed from random expectations. (Online version in colour.)

Although Williams & Martinez [40] were able to assign 54% of 212 species in four food webs unambiguously to a discrete TL, Thompson *et al.* [23] found omnivory to be widespread above the herbivore TL. Given the prevalence of omnivory, we estimated a discrete max and min TL for a parasite’s definitive host. For the host’s max TL, we added one to the TL of its highest TL food item. Similarly, for min TL, we added one to the TL of its lowest TL food item. In both estimates, the dietary food item TL was scored conservatively. Strict herbivores thus had a min TL of 2 and maximum of 3 to reflect the fact that they accidentally ingest other primary consumers (e.g. an herbivorous insect eaten by a grazing mammal).

After estimating min and max TL, we estimated an average TL for each species on the basis of information about host diet. For instance, herbivores acquire nearly all their energy from primary producers and only a fraction by accidentally eating primary consumers, so their average TL estimate was 2. Diet information for mammals was taken from Jones *et al.* [41], and for fishes mean TL estimates were taken from FishBase [39]. For other taxa, diet information was taken from a wide range of sources and where no additional information was available the average of min and max TL was taken as the best estimate of average TL. Although some TL estimates are more precise than others, this does not seem to introduce bias. Preliminary analyses that used only weighted TL estimates (mostly from FishBase) or only ‘rougher’ estimates based on discrete TLs returned very similar results. Many nematodes can infect several species as definitive hosts, so we averaged the different host TL estimates to give a single value for each nematode species. The different definitive hosts used by a given nematode species tend to have similar trophic ecologies. In summary, our dataset consisted of two measures for LCL (long and short) and three for definitive host TL (minimum, maximum and average).

## (b) Establishing a phylogeny for analyses

Given their shared history, LCLs and host TLs for different species are not necessarily independent in the statistical sense, so a phylogeny must be incorporated into the comparative analysis [42,43]. No available phylogenies encompass all of the nematodes with deduced life cycles. Thus, we took the phylogeny from van Megen *et al.* [44] as a backbone tree, with branch lengths, and we added species to it based on available phylogenetic and taxonomic hypotheses (see the electronic supplementary material). The internal node for species or clades added to the tree was arbitrarily placed at the halfway point on the branch leading to their sister taxa, and the internal branches of clades added to the tree were assumed to have equal lengths. Polytomies were used in

the case of uncertainty and the tree was scaled to be ultrametric for use in statistical analyses.

## (c) Association between definitive host trophic level and life cycle length in trophically transmitted nematodes

We defined max TL as the longest expected trophic chain leading to a given definitive host. Max TL therefore sets an upper boundary for the number of hosts and trophic transmission events in a life cycle (figure 1a). In our dataset, this constraint is  $LCL \leq \max TL - 1$ , because trophically transmitted, animal–parasitic nematodes begin their life cycle by infecting an animal consumer (at least TL 2), not primary producers like plants. For example, a parasite that infects a definitive host with a max TL of 3 can have a maximum LCL of 2, i.e. a primary consumer as first host (TL = 2) and a secondary consumer as definitive host (TL = 3). On the other hand, the min TL of a host does not, by definition, impose a constraint on LCL (figure 1b). Even hosts with a high min TL could be infected directly, assuming parasite propagules can be accidentally ingested.

We performed multiple regression analysis with min and max TL as independent variables and LCL as a dependent variable. However, LCL and max TL are expected to be positively correlated, even if the data are randomly distributed in the ‘samplable’ phenotype space (i.e. long life cycles can only be observed when max TL is large; figure 1). The normal null hypothesis that regression parameters equal zero is thus unsuitable. We performed permutations to establish the null parameter distributions, but the permutations cannot be completely random given the constraints in the data. For instance, a high LCL randomly paired with a low max TL is implausible, as this falls outside the observable phenotype space. We structured the permutation procedure. Parasites with long life cycles are the most restricted in the TL values their definitive hosts can have, so we started by assigning random, but constraint-satisfying TL values to parasites with long cycles. For instance, a parasite with four hosts in its life cycle can only plausibly include a definitive host with a max TL of 5 or more (figure 1). All data points where max TL equals 5 or more were isolated and then randomly paired with species that have LCL = 4. The remaining, unassigned TL values were then used in the next case, LCL = 3. Here, max TL must equal 4 or 5, so all data points fitting this criterion, excluding those already assigned for LCL = 4, were isolated and randomly paired with species that have LCL = 3. This was continued until all TL values had been randomly assigned.

This structured permutation randomly combines observed LCL and TL values within the confines of the ‘samplable’

**Table 1.** Results of phylogenetic regressions with LCL as a dependent variable and min and max TL of the definitive host as independent variables. (We assessed LCL twice, once assuming nematodes with flexible life cycles have the longest possible cycle and once assuming they have the shortest possible cycle. A positive slope for max TL was expected by chance (figure 1a), so the null distributions were established by a structured permutation procedure, in which the LCL values were randomly paired with constraint-satisfying TL values. *p*-Values in bold were considered significant.)

dependent variable	regression parameter	expected (range in which 95% of permutations fall)	observed	<i>p</i> -value
short LCL	intercept	1.425 (1.238–1.610)	1.053	<b>0.001</b>
	min TL	0.008 (–0.078 to 0.088)	0.190	<b>0.001</b>
	max TL	0.059 (0.003–0.109)	0.058	0.987
long LCL	intercept	0.895 (0.691–1.085)	0.476	<b>0.001</b>
	min TL	0.017 (–0.102 to 0.132)	0.227	<b>0.001</b>
	max TL	0.235 (0.168–0.302)	0.232	0.931

phenotype space (figure 1), and it has several advantages. First, the mean and variance of all variables (LCL, min TL and max TL) are unchanged. Second, min and max TL are correlated, and because these variables are permuted and assigned simultaneously, the procedure does not break this correlation. Third, the phylogenetic structure in LCL is maintained as only TL is permuted. The structured permutation was repeated 999 times, and each time a phylogenetic regression [45] was conducted with the permuted dataset to establish the null distribution for the regression parameters. Phylogenetic signal in the residual error term (Pagel's  $\lambda$ ) was estimated simultaneously with the regression parameters [43] using the *ape* R package [46]. Pagel's  $\lambda$  ranges from 0 (no phylogenetic signal) to 1 (strong phylogenetic signal). The structured permutation was conducted with both LCL measures (long and short) as a dependent variable.

We also performed a phylogenetic regression simply using average host TL as an independent variable. Here, we tested the normal null hypothesis (regression parameters = 0), because the average TL does not necessarily constrain LCL, i.e. even a host with a low (or high) average TL can still have long (or short) trophic chains leading to it.

#### (d) Comparison with alternative transmission strategies

Besides trophic transmission, two other transmission strategies are widespread in nematodes infecting vertebrates: penetration and vector transmission [31]. Penetrators have a one-host life cycle in which they penetrate through the skin of their host and then migrate to another site for reproduction. Vector-transmitted species have two-host life cycles; they are transmitted to a vertebrate definitive host via the bite of a blood-feeding vector. Parasites with these transmission strategies are not constrained by the food web, so it is interesting to ask whether, compared to trophic transmission, either of these alternative strategies allows parasites to reach high TL definitive hosts with fewer hosts in the life cycle.

To compare transmission strategies, we fitted two alternative phylogenetic models based on the Ornstein–Uhlenbeck process. This process involves random fluctuations around an optimal phenotype and is taken to represent stabilizing selection [47,48]. In the first model, we only estimated a single optimum for definitive host TL, irrespective of transmission strategy. In the second model, we estimated separate optima for host TL for five different transmission strategies: (i) trophically transmitted with one host ( $n = 93$ ), (ii) trophically transmitted with two hosts ( $n = 228$ ), (iii) trophically transmitted with three or more hosts ( $n = 71$ ), (iv) penetration ( $n = 30$ ) and (v) vector transmission ( $n = 115$ ). Trophically transmitted worms were classified into these groups using their longest possible LCL. Similar results were obtained when using the short version of

LCL, so for simplicity only results using long LCL for classification are presented. Models were fitted with the OUCH R package, and they were compared with likelihood ratio tests [48].

All statistical analyses were conducted in R 2.15.1 (R Development Core Team, Vienna, Austria).

### 3. Results

#### (a) Definitive host trophic level and life cycle length in trophically transmitted nematodes

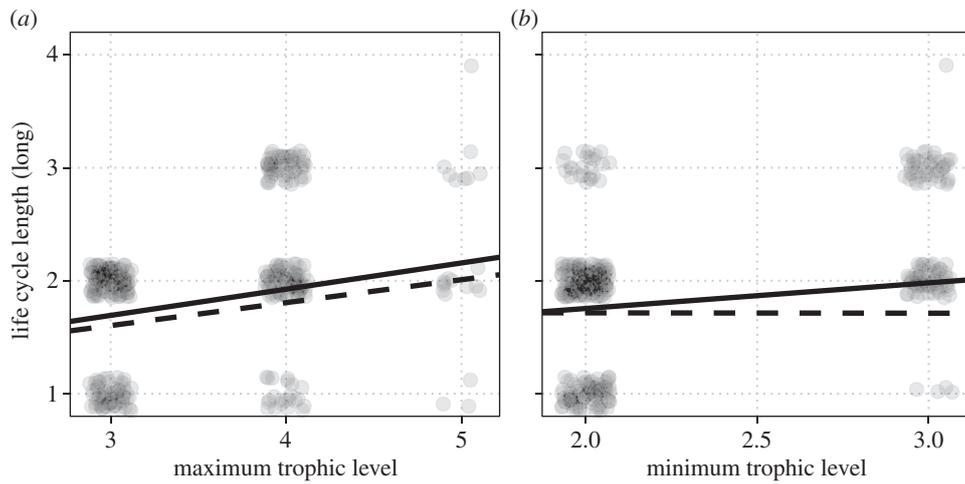
LCL is constrained to be less than the max TL (figure 1), and the permutation procedure accounting for this indicated that LCL increased with max TL, but not more than expected by chance. However, LCL increased significantly with min TL (table 1 and figure 2). Nematodes with long life cycles usually reproduce in definitive hosts with significantly higher average TLs (short measure of LCL =  $0.17 \times \text{TL} + 1.20$ , Pagel's  $\lambda = 0.96$ , *p*-value for slope < 0.0001; long measure of LCL =  $0.35 \times \text{TL} + 0.82$ , Pagel's  $\lambda = 0.94$ , *p*-value for slope < 0.0001) (figure 3), though note that these slopes are weak compared with the 'perfect' slope of 1.0.

#### (b) Alternative transmission strategies and host trophic level

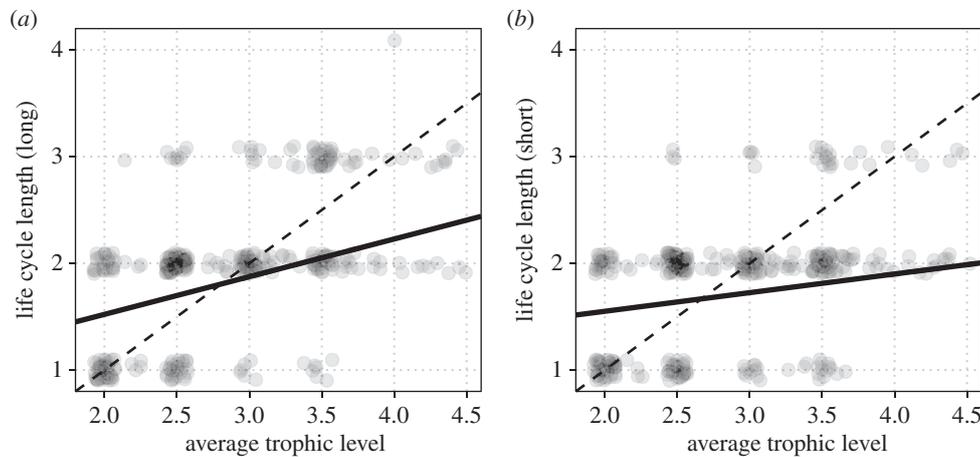
Definitive host TLs differ among nematode transmission strategies (single optimum model versus separate optima model, likelihood ratio tests: max TL,  $\chi^2_4 = 53.86$ ,  $p < 0.0001$ ; min TL,  $\chi^2_4 = 67.31$ ,  $p < 0.0001$ ). To compare the strategies, confidence intervals (CIs) around the mean for each transmission strategy were estimated by bootstrapping the model [48] and are plotted in figure 4. Compared with trophically transmitted worms with one host, penetrating worms have definitive hosts with significantly higher TLs (i.e. means fall outside the CIs). Vectored worms, on the other hand, have definitive host TLs that are comparable to (for max TL) or slightly lower (for min TL) than trophically transmitted worms with two hosts.

### 4. Discussion

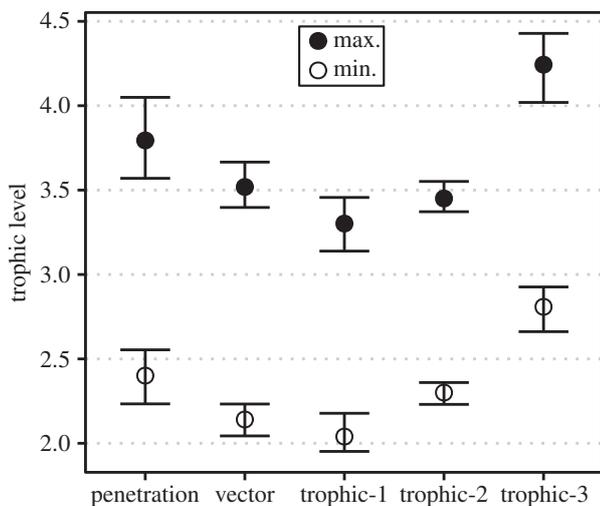
High TL predators tend to be large and long-lived [9], so they are assumed to be excellent hosts for parasites, offering high growth and low mortality prospects [19]. However, parasites in high TL hosts may be unable to produce propagules that



**Figure 2.** LCL as a function of the (a) max and (b) min TL of the definitive host. Data points were randomly jittered to reduce overplotting and improve visualization. The solid lines are based on a phylogenetic, multiple regression model. The dashed lines are the null expectations formed on the basis of structured permutations (see text). The observed regression differs significantly from the null in (b) but not (a). The long version of LCL is depicted, though the short version displayed a similar pattern.



**Figure 3.** Nematode LCL, long (a) or short (b) version, plotted against the average TL of the definitive host. Data points were randomly jittered to reduce overplotting and aid visualization. Solid lines are the relationships predicted by phylogenetic regression. They were statistically significant in both cases. Dashed lines depict the hypothetical case in which LCL increases by one host for each increase in definitive host TL, i.e. if parasites are transmitted through all hosts in the typical trophic chain leading to a definitive host.



**Figure 4.** The min and max TL of the definitive host for nematodes with different transmission strategies: direct penetration, vector transmission or trophic transmission of life cycles of 1, 2 or 3 hosts in length (for flexible cycles; longest cycle length was used). Bars represent 95% CIs estimated by parametric bootstrapping of Ornstein–Uhlenbeck models.

are trophically available to those hosts, i.e. the propagules fall into a transmission vacuum. There has been little attempt to empirically demonstrate or quantify such transmission barriers. Here, we show for the first time that nematodes reproducing in high TL hosts tend to have more successive hosts in their life cycles. This suggests that either infecting top predators favours the incorporation of intermediate hosts into the life cycle (downward incorporation, *sensu* [19]) or that adding top predators to a cycle requires retaining hosts at lower TLs (upward incorporation, *sensu* [19]). While the results are consistent with a trophic vacuum, from our analysis we cannot determine which mechanism (upward or downward incorporation) has been more important in producing this pattern.

If parasite transmission were constrained by a perfect trophic vacuum, we would predict that herbivores (TL = 2) are infected directly (LCL = 1) and with each step up the food web, LCL increases by one. The observed relationship between LCL and average host TL was higher and shallower than this prediction (figure 3). This implies that nematodes reproducing in low TL hosts had surprisingly long life cycles, e.g. the phylogenetic regression predicted worms infecting herbivores (TL = 2) to have on average approximately 1.5 hosts in

their life cycle. Metastrongylids illustrate this. These worms typically use terrestrial molluscs as intermediate hosts, which are then accidentally consumed by a grazing ungulate [31]. Here, the intermediate host does not transport parasites to a higher TL, given that an herbivore is incidentally consuming another herbivore. The intermediate host could serve other functions, such as increasing the survival, longevity and/or dispersal of infective stages [1,20,49]. These worms are transmitted from a small to a large host, which highlights the fact that TL and body size are imperfectly correlated [9]. As a consequence, it may be possible for trophically transmitted parasites to infect large, but low TL hosts and enjoy the presumably better conditions for growth and reproduction [19], without necessarily having longer life cycles.

Although significantly positive, the slope of the LCL–TL relationship was modest (0.35 for the long version of LCL, 95% CI: 0.27–0.44). Strong phylogenetic structuring often reduces slope estimates in phylogenetic regressions [43], and the high values of Pagel's  $\lambda$  (greater than or equal to 0.94) indicate that related nematodes tend to have similar combinations of LCL and TL. However, the non-phylogenetic least-squares estimate of the slope was 0.56, suggesting that the shallow slope (less than 1) is not entirely a by-product of phylogeny. This is not consistent with a pervasive trophic vacuum, and it suggests some parasites can move up the trophic web without transmission through each link in the average trophic chain leading to their definitive host. *Ollulanus tricuspis* is a tangible example. This worm reproduces in felids, including very large ones like lions and cougars, where it causes irregular vomiting that releases the larvae [50]. Transmission to other cats occurs when they consume the vomit.

The notion of a trophic vacuum assumes that parasite propagules occupy a low TL and are primarily available to small, low-level consumers. However, with larger eggs or larvae, parasites could enter the trophic chain at higher levels, reducing the number of steps needed to reach top predators. For instance, in trematodes the host-seeking cercarial stage is bigger in species that target vertebrates, as opposed to invertebrates [51]. Parasites might also shorten the life cycle by making low TL intermediate hosts more profitable prey for high TL predators, e.g. by manipulating intermediate host behaviour [24]. Food web studies suggest that parasites can strengthen existing trophic interactions [52] and forge new links between host species (or at least uncover previously undocumented interactions) [53–56]. Though parasite adaptations like propagule structure or host manipulation may reduce the barriers to trophic transmission, they do not eliminate them. Nematodes reproducing in exclusively carnivorous hosts (high min TL) have on average more hosts in their life cycles, suggesting that there are limits as to how much parasites can facilitate unlikely trophic events [57].

The significant relationship between LCL and min TL also suggests that the short trophic chains leading to a host define the boundaries of the trophic transmission vacuum. Animals frequently feed on multiple TLs [23,58], and this is particularly true for large, high TL animals [59,60]. This gives trophically transmitted parasites the opportunity to preferentially exploit the short trophic chains leading to a host. Besides the obvious benefit of shortening the life cycle, parasites may have higher transmission rates along shorter chains, as energy flows are thought to be greater along these chains [40,61]. This is likely true even if energy flows to a predator are equal among chains. For example, consider a predator that gets half its

energy from consuming herbivores (TL = 2) and half from secondary consumers (TL = 3), so that its TL is 3.5. Presuming the herbivore is smaller than the consumer [9], the predator would consume more low TL prey items than high TL items to meet its energy demands. Thus, in this hypothetical case, a parasite would have a higher transmission rate along the short chain, because there are more consumption events, even though energy flows are the same along the two chains. An additional consideration is that shorter chains may be more dynamically stable over time [62–64], and stability is a characteristic of parasitized trophic links [65–67].

Not all nematodes take an oral route to their final hosts; penetration and vector transmission are two alternative transmission strategies. Penetrators have a direct, one-host life cycle, and compared to trophically transmitted species with one-host cycles, they infect higher TL definitive hosts. This is perhaps expected since trophically transmitted nematodes with one host are generally constrained to exploit low TL hosts, even though allogrooming or omnivory by higher predators may allow direct transmission. Penetration, though, seems to allow parasites to reach high TL hosts without multiple hosts. A caveat here is that our analysis focused exclusively on vertebrate parasites, ignoring nematode species that penetrate, kill and reproduce in invertebrates [68,69]. Including these species would certainly reduce the average TL for the penetration strategy.

By contrast, vector-transmitted nematodes have a two-host life cycle, but on average they do not reproduce in higher TL hosts than trophically transmitted species with two-host cycles. In vector-borne nematodes, the parasite alternates between the lower TL of its definitive host and the higher, micro-predatory TL of its vector, which is the opposite of trophically transmitted parasites that move up TLs to reach the definitive host. It nevertheless satisfies the idea that a smaller host (the vector) is necessary to access a large definitive host, which is consistent with the basic premise of the transmission vacuum. It must be kept in mind that nematode transmission strategies differ in a number of respects (e.g. vector-transmitted nematodes reproduce in the tissues and not the gut, penetrators are basically terrestrial, etc.). Consequently, the ultimate causes for the evolution of different transmission strategies are likely multitude [70], with definitive host TL and the associated obstacles with oral transmission being just one presumably important aspect. Additional studies contrasting these strategies would thus be worthwhile.

In conclusion, our results suggest that trophically transmitted parasites are confronted with a transmission barrier between non-adjacent TLs. To reach high TL hosts and bridge this trophic vacuum, parasites typically use one or more intermediate hosts and have longer life cycles. Other transmission strategies, like penetration, are not subject to this constraint. However, the trophic vacuum may be weaker than often assumed, as it is determined chiefly by the shortest trophic chains leading to a host (i.e. the min TL). Both ecological factors (omnivory) and parasite adaptations (propagule size/structure, host manipulation) probably play a role in reducing, but not eliminating, trophic transmission barriers.

**Data accessibility.** Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.5bd66>.

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