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Ecological study of the micropredator, *Taimenobdella amurensis*, a piscivorous leech: filling the gap between predation and parasitism

（魚類捕食性エゾビルの生態学的研究：

捕食と寄生の間を埋めるマイクロプレデーション）

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SUMMARY

Predator-prey and parasite-host relationships are ubiquitous in nature and have been extensively studied in the fields of ecology and evolution. The main difference between predators and parasites are the number of victims: individual predators kill many prey throughout their life, while parasites usually spend their entire or each stage of their life within a single host, which does not necessarily result with host death. However, there is another trophic strategy in between, which has been neglected. These are “micropredators”, animals such as mosquitoes and vampire bats, that consume only a part of the prey’s body without killing it, but requires multiple prey throughout their life. Some studies have clearly separated micropredation from other trophic strategies and pointed out the importance of treating them separately, but the concept of micropredation has been underappreciated or even misused. Studies on micropredator-prey relationships from different aspects are certainly needed.

In this PhD thesis, I firstly conducted a literature review focusing on how the term and concept have been used since its first appearance in the 1970’s. The original term used for this trophic category is as mentioned above, but later it has also been used for small (i.e. micro) predators, like bacteria eating other bacteria. The terminology has been greatly

underused even after a seminal review paper in 2002 that clearly categorized different trophic strategies. I listed a variety of taxa that fall into the category of micropredator based on the defined criteria, and found that micropredators exist within diverse animal groups, such as Ixodida, Siphonaptera, Diptera, Lepidoptera, Piscicolidae, lampreys, vertebrate fishes, birds, and mammals, suggesting the parallel evolution or convergence of the strategy. In the literature they were rarely referred to as micropredators: rather, some of them (e.g. lampreys and leeches) were often called “parasites”, while the most representative groups (i.e. mosquitos and ticks) were called “vectors”. In fact, many micropredators are haematophagous (blood-sucking) and have significant impacts on ecosystems through the transmission of diseases. Their co-evolutionary history is weak compared to parasite-host relationships, but seems to be stronger than predator-prey relationships. I noted that the reason for the relative ignorance of micropredators is partly due to the lack of clear examples, especially model systems.

I then provide a case study of the micropredatory leech, *Taimenobdella amurensis*, and their main prey fish, *Salvelinus curilus*, in the Sorachi River, Hokkaido, Japan. Since the metapopulation dynamics and genetics of the prey fish have been studied for more than 20 years, it is ideal for investigating micropredator-prey relationships. Here, I specifically focused on the genetic structure of the leech, testing if they mirror parasite-host co-structures or show independent structure from their prey, despite depending on dispersal via prey fish. Thirteen microsatellite primers were designed specifically for this leech. Population genetic analysis showed extremely high genetic divergence even among neighboring streams, without any pattern of isolation-by-distance. This contrasted with the genetic structure of the prey fish and suggested that the functional dispersal by attaching to their prey is minimal. This also indicates that these leeches can form independent local populations even with very small population sizes. Thus, even with the seemingly long periods of attachment and prey specificity, this micropredatory leech does not form a parasite-host like relationship with prey fish: rather, a predator-prey like free-living model is more suitable.

Finally, I examined the generality of micropredator-prey genetic structure by reviewing past literature. No clear effect of trophic strategy (i.e. micropredators, parasites, and free-living animals) on genetic divergence was detected. This was partly due to large variations within trophic strategies: for example, some terrestrial micropredatory leeches showed very strong genetic divergence, which is consistent with my study, but another species that attaches to the eyes of seabirds was genetically homogenous, because of long-distance dispersal via avian migration. The taxonomic differences between species used to represent different trophic strategies was likely also a confounding variable. More critically, there were too few case studies to confirm the general patterns.

Overall, I concluded that micropredators are relatively common, though not as common as predators and parasites, in many ecosystems. They have unique ecological characteristics, sometimes shared with either parasites or predators. We should incorporate and distinguish them as a legitimate ecological strategy and I hope this case study of the leech-fish system inspires studies on other micropredator-prey systems.

GENERAL INTRODUCTION

Predator-prey and parasite-host relationships are ubiquitous in nature and have been extensively studied in the fields of ecology and evolution (Lafferty 2008; Lafferty et al. 2015). The main difference between predators and parasites are the number of victims: individual predators kill many prey throughout their life, while parasites usually spend their entire or each stage of their life within a single host, which does not necessarily result with host death (Lafferty and Kuris 2002; Lafferty 2008). However, there is another trophic strategy in between, which has been neglected. These are “micropredators”, animals such as mosquitoes and vampire bats, that consume only a part of the prey’s body without killing it, but requires multiple prey throughout their life (Lafferty and Kuris 2002; Lafferty 2008). Some studies have clearly separated micropredation from other trophic strategies and pointed out the importance of treating them separately (Lafferty and Kuris 2002; Lafferty 2008; Lafferty et al. 2015), but the concept of micropredation has been underappreciated or even misused. Studies on micropredator-prey relationships from different aspects are certainly needed.

Compared to micropredators, parasitoids (animals that require the death of their host as part of their lifecycle, such as parasitoid wasps) are a well established and documented group of organisms, despite being largely restricted phylogenetically to only a few phyla (i.e. Arthropoda and Nematomorpha) (Whitfield 1998). Yet, even with the numerous similarities to parasites, parasitoids are always carefully separated from other kinds of parasitic lifestyles, and never included with classical parasites such as the helminths. The term micropredator covers a much broader range of species within the kingdom Animalia, possess a number of traits that distinguish them from parasites, and in many respects are much closer to predators, but are nevertheless often grouped with parasites. This is particularly interesting in that parasitologists have broken the broad consumer strategy of parasitism into many different subdivisions (trophic parasites, parasitic castrators, brood parasites, parasitoids, ect.) and yet the strategy employed by micropredators is rarely described or even used in academic literature.

The term "micropredator" is not particularly popular in ecological research, used in several disciplines of ecology since the mid-20th century, it has never seen much use in the scientific literature. Despite this, in common parlance it has begun to receive some traction among parasitologists as a casual way of describing organisms that fall into a grey-zone between parasites and predators (Lafferty et al. 2015), though are often lumped in with parasites regardless of well they fit the description.

In this thesis, I provided a comprehensive review of micropredation and a case study of the micropredatory leech, *Taimenobdella amurensis*. Lafferty and Kuris (2022) succinctly defined micropredator, but just as one of the many trophic strategies and did not elaborate further on its application. I firstly reviewed the history of the terminology to see how the term has been used in scientific literature, and I found it was generally underused or misused. Then, I listed the animal taxa that fit the characteristics of a micropredator. I found that micropredation has evolved in different clades at multiple times and discuss the evolutionary origins of the trophic strategy and co-evolution with their prey. I finished the review on the ecological roles of micropredators, such as vectors and density-dependent impacts on their prey. In the next chapter, I provided a case study of the micropredator-prey relationship between the piscivorous leech *Taimenobdella amurensis* and the main salmonid prey, *Salvelinus curilus*, in the Sorachi River, Japan. Since the metapopulation dynamics and genetics of the prey fish have been studied for more than 20 years, it is ideal for investigating micropredator-prey relationships. Here, I specifically focused on the genetic structure of the leech, testing if they mirror parasite-host co-structures or show independent structure from their prey, despite depending on dispersal via prey fish. In the last chapter, I examined the generality of micropredator-prey genetic structure by reviewing past literature and cover the implications of micropredation on the formation and maintenance of their populations in the general discussion. I concluded that micropredators are relatively common in many ecosystems and play significant roles, and therefore we should incorporate and distinguish them as a legitimate ecological strategy.

Chapter 2: Comprehensive review on micropredation: definition, history, evolution, and ecological significance

Definition of predators, parasites, and micropredators

The ecological traits that define micropredators and separate them from other types of consumer strategies, have already been succinctly explained in previous papers on the subject. The best comparison to date is Lafferty and Kuris (2002), in which they outlined 4 dichotomies capable of separating different "enemy-victim" relationships as defined by their trophic strategies, resulting in seven types of parasitism and three types of predation (Fig.2-1; Lafferty and Kuris 2002). This definition has been widely accepted in scientific literature (Poulin and Randhawa 2015) partly due to the unambiguousness of the dichotomies compared to the relative scale, such as intimacy to the hosts. The authors later constructed a general consumer-resource model based on the trophic strategies, as well as other consumer strategies, such as autotroph, decomposer, and detritivore (Lafferty et al. 2015).

Micropredators were categorized as a predator by this metric, and along with "solitary predators" and "social predators" constituted one of the three predatory consumer strategies discussed in Lafferty and Kuris (2002). The dichotomies they outlined were as follows:

1) does the enemy attack more than one victim

2) does the enemy eliminate victim fitness

3) does the enemy require the death of the victim

4) does the enemy cause intensity-dependent pathology

In the first dichotomy, Lafferty and Kuris (2002) further elaborate that feeding on multiple victims per life-stage is a key distinction, as many parasites, such as the digeneans, have multiple hosts over the course of their lifecycles but only one per each stage in their development. Micropredators by contrast, can generally attack multiple victims during each stage of their lifecycles, a similarity they share with other predators. Female mosquitoes for example, feed on vertebrate blood only during the winged adult stage of their lifecycle and will attack as many prey individuals as is necessary for the production of eggs (Krenn and Aspöck 2012).

The second and third dichotomies are similar for most consumer strategies, but have a key difference for animals that engage in parasitic castration of their hosts, which reduce host fitness to zero but do not kill them. However, micropredators require neither the death of their prey nor do they reduce prey fitness to zero, a trait they share with parasites and that separates them from other predators, which generally require the death of their prey, and therefore also reduce prey fitness to zero. This is in fact their defining characteristic as predators, as the "micro" in "micropredator" is not referring to the size of the predator, but the relative amount consumed by the predator in comparison to its prey's mass (Lafferty et al. 2015).

The fourth dichotomy separates micropredators from other kinds of predators and parasites as well, which all generally have clear delineations between intensity dependent and independent pathology categories, for example solitary vs social predators or parasites vs parasitoids (Lafferty and Kuris 2002). In micropredatory species, intensity dependent or independent pathology is the result of the relative size of a micropredator to its prey, and in some taxa this can verge on facultative predation. *Hirudo medicinalis,* feeds on the blood of a wide range of vertebrates and a single leech is rarely a concern for a human, but the same leech is pathological on an amphibian (Merilä and Sterner 2002). Relative size aside, as the number of micropredators feeding on an individual increases, so too does the impact on prey health, and the health implications swarms of biting flies or tick infestations have are well documented in wildlife and domestic animals (Musante et al. 2007; Smith et al. 1998)

Thus, uniquely based on these dichotomies pathology in micropredators is neither strictly intensity dependent or independent.

These 4-dichotomies effectively separates different consumer strategies and some additional characteristics become clear. For example, a similar characteristic between predators and micropredators, but not parasites, is the lack of lethality for failure to attack their prey. If a predator or micropredator fails in an attack they are not likely to die and may even attack the same individual multiple times, whereas if a parasite is unable to successfully attack a suitable host during its dispersal phase, it is likely to die (Lafferty et al, 2015).

Lafferty and Kuris (2002) defined herbivores as a subgroup of micropredator that preys on sessile organisms. This group has a number of critical differences compared to micropredators of motile organisms. First, the relative body sizes of micropredators of motile prey are much smaller ($< 10^{-2}$) than their prey, probably resulting from the adaptation to prey detection and crypsis, whereas the relative body sizes of herbivores to host plants can take any value from mite-to-tree or cow-to-herb. The main purpose of Lafferty and Kuris (2002) was to explain the different trophic strategies based on consumer-victim relative body size: in this sense, micropredators on sessile prey or herbivores are exceptional. Second, because sessile prey cannot escape from micropredators spatially, sessile prey have developed a variety of anti-micropredator strategies, such as chemical and physical defenses (Johnson 2011). This also creates an arms race between micropredators and prey, especially small arthropods that often exhibit specific adaptation or speciation to a single prey plant species. This level of co-evolution differs significantly from prey-micropredator relationships on motile prey, as will be discussed below. Third, micropredators, by definition, consume only a small part of their prey, but herbivores often eat whole individuals, especially large mammals that eat annual plants. In this case, the delineation between predation and micropredation is ambiguous. Overall, micropredators of sessile prey possess different ecological/evolutionary characteristics, have been extensively studies as herbivores, and can be clearly separated, I will focus mainly on micropredators of motile prey in this thesis and only refer to them when necessary.

What taxa are best described as Micropredators

Given the definition above, a wide range of species can be classified as micropredators of motile prey (Table 2-1). Haematophagous animals generally fall into the category of micropredator as they often do not require the death of their prey, are generalists, smaller than their prey, and are unaffected by failed attacks. Interestingly, haematophagous has arisen from many different animal taxa, such as Ixodida, Siphonaptera, Diptera, Lepidoptera, Piscicolidae, fishes, birds, and mammals. By number of species, most micropredators can be described as haematophagous.

Another group of micropredators are those that consume chunks of prey individuals. This includes species like some amphipods and isopods, nudibranchs, lamprey, cookie-cutter sharks, and scale- or fin-eating fishes. Scale- and fin-eating, lepidophagy and pterygophagy respectively, is a specialized forms of micropredation in fishes and has evolved independently at least 5 times among freshwater families and seven times among marine families (Janovetz 2005; Lavoué et al. 2017). Due to being a specific feeding mode, scale- and fin- eating fish are not generally mixed in with parasites, making it far easier to determine the phylogenetic extent of this feeding strategy than micropredation in isopods, amphipods, or nudibranchs. Isopods for example, are generally free-living, with only seven parasitic or micropredatory families, mostly on fish, of which the Gnathiidae are well for their micropredatory feeding behaviour (Poulin 1995; Smit et al. 2014). But, the number of micropredators among the amphipods and isopods is currently difficult to correctly assess, as many scavenger or carnivorous species have very diverse feeding habits, and so may also act as micropredators (Blankenship and Levin 2007). Cleaner fishes and ox-pecker can also be categorized as micropredators: they are usually symbionts, eating parasites of host individuals, but opportunistically feed on skins, hair, feather, blood, and scar tissue of prey species. In some fish such as the wrasses, this has led to the evolution of so called "false-cleaners" which mimic the appearance and behaviours of cleaner wrasses, and feed primarily on prey fin tissue (i.e. micropredator).

A variety of detritivourous and pollinating insects such as many Diptera and Lepidoptera, will also feed on the fluids of vertebrates when available in order to acquire nutrients that are rare or lacking in their regular diet (Krenn and Aspöck 2012). This could also be described as micropredation; however, as it only arises from opportunity these species might not strictly be micropredators, though they are engaging in that form of feeding. Similarly, predatory animals that do not always kill their prey are fairly common, many species will stop pursuit if their prey drops an appendage while fleeing, such as lizards dropping their tails, or arthropods shedding limbs. Such behaviour is not micropredation, as it is a result of prey behaviour and not the predator's, and the prey not being killed and consumed is an unsuccessful attack for the predator.

Use in the literature

The earliest use of the term "micropredator" we could identify appears in scientific literature from the 1940s (Fig. 2-2), where it was used to differentiate between predatory microbes and microbial prey within soil microbe communities, as well as to refer to predators of microbes more generally. Interestingly, according to Google Scholar search results, no other cases of the term appear again until the 1960s when it is used to describe animals like haematophagous flies or marine crustaceans, similar to how it is defined in this manuscript. In the 1970s we begin to see the term's use expand to include predators of small organisms such as filter feeders, or small predators like the predatory land snail *Gulella bicolor* (Hutton) and predatory protozoans.

To determine how the term is used, and how it has trended in the scientific literature, I collected search results for all incidents of the term "micropredator" from its earliest use until the end of 2019 on Google Scholar and SCOPUS, retrieving only 561 and 37 papers, respectively. One of the reasons for the very low result count with SCOPUS in particular, was that it allows searching by title, abstract, and key words. This greatly reduced the chance of counting the same papers multiple times through references, or finding obscure in-text occurrences of the term, which were common issues when sorting the term usage on Google Scholar. In my Google Scholar search results there were several notable topics where the term "micropredator" was frequently used, such as the search terms "Antarctica micropredator" of which 318 results of 553 were related to the usage we employ in this paper. A related search using the terms "gnathiid isopod and micropredator" resulted in 142 of 215 references applying the definition we use in this paper. While the image of a micropredator is often a mosquito or tick, in the literature marine crustaceans are far more likely to be referred to as micropredators, particularly amphipods and isopods. The predatory land snail *Gulella bicolor* (Hutton) was the most common species referred to as a micropredator, as in small predator, with 13 and 17 results of 139 small predator references I found for the search terms "*Huttonella* and micropredator" and "*Gulella* and micropredator", respectively.

How have micropredators been called?

Looking at a variety of species that can fall into the trophic categoury of micropredators, and searching by their common names and "micropredator" we determined what other terms were being used to describe them in literature. "Predator", "parasite", and "vector" were also paired with the common names of these species, and it became apparent that the two most common descriptors were "parasite" or "vector". Due to the feeding habits of micropredators, many of them act as vectors for disease in humans and other animals, but that is a consequence of their feeding habits and not an ecological strategy itself. Many of the species I used for web-search are harmful vectors for diseases or disease causing in other ways (infestation), as a result there have been thousands of studies on them, particularly in efforts to control or reduce the damage they cause. Ticks, fleas, mosquitoes, and other biting flies had thousands of results for "vector" or "parasite", while "micropredator" never exceeded more than 30 results (Table 2-2). The only search terms that had a significant proportion of its results for "micropredator" were "Gnathiid Isopods", which had 8% on SCOPUS and 13% for Google Scholar results, but had fewer results in general than most of the animals I searched (Table 2-3). The species I used that are not known to spread diseases or cause infestations were much less studied, and often had no search results when combined with "micropredator".

Some species such as lamprey, were frequently described as "parasitic" in the literature despite having little similarity with parasites. In fact, given the high prey mortality during or after feeding, frequently exceeding 50% prey mortality (Bence et al. 2003), lamprey are an example of how micropredator pathology is strongly influenced by relative prey size. The only similarities lamprey had with parasites were their long attachment time to prey, and the lack of necessity in reducing prey fitness to zero. Though sources on species like cookie-cutter sharks were rare in general, they were almost unanimously referred to as "predators" in the literature; while they are known to predate on small squid and fish, the evolutionary adaptations to their jaws and behaviour clearly imply that micropredation is their main form of feeding (Papastamatiou et al. 2010; Pérez-Zayas et al. 2002).

Evolution of micropredation and prey responses

It is clear from the existence of micropredators from diverse taxa (Table 2-1) that this trophic strategy has been evolved many times, independently from different lineages. Lafferty and Kuris (2002) emphasized the importance of relative body size between the enemies and victims in different trophic strategies, but here I more carefully examine micropredation focusing on its evolutionary origins.

Given the definition of micropredation (i.e. consume tiny parts of the prey) and relative body size, the ancestor of micropredators should be predators and, then, the evolution of size divergence between predator and prey resulted in micropredation. This may be the case for the micropredators that consume chunks of their prey, such as some types of marine isopods, scale-eating cichlids, and false-cleaner fishes. Cookiecutter sharks (*Isistius* spp.) in particular are an illustrative example for the evolution of size divergence to better utilize this type of feeding strategy. When compared to their nearest relative, the kitefin shark (*Dalatias licha*)(Claes and Malefet 2009), cookiecutter sharks have a noticeable reduction in body size. It is also interesting to note that while kitefin sharks also engage in micropredatory feeding opportunistically, they are less specialized morphologically and behaviourally than cookiecutter sharks for this hunting strategy (Grace et al. 2019; Soto and Mincarone 2001).

Feeding habits, rather than body size, should be the major driver for the evolution of haematophagous micropredation. The ancestors of blood-feeders are often free-living pollinators and frugivores (Krenn and Aspöck 2012; Zaspel et al. 2014). The origin of blood-feeding in vampire bats (family Phyllostomidae) for example, is generally agreed upon to have come from a dietary shift, possibly from a fruit eating or ectoparasite eating ancestor (Fenton 1992). Haematophagy in mammals is an extremely unusual feeding mode, only three obligate blood-feeders, due to the adaptations necessary not just to acquire but to digest it (Fenton 1992; Mendoza et al. 2018). By comparison, in practically all clades of arthropods that possess sucking mouthparts some form of blood-feeding behaviour has evolved, and in most of these micropredation is the most common feeding strategy, with lice (Anoplura) standing out as obligate ectoparasites (Krenn and Aspöck 2012). Male moths of the family Noctuidae, for example, are known to occasionally feed on mammalian blood using their mouthparts which are adapted for piercing thick-skinned fruits, and functionally little change is required to switch between the two food sources (Krenn and Aspöck 2012; Zaspel et al. 2014). The Hemiptera are an order of insects comprising over

50,000 species that all possess sucking mouthparts (Song et al. 2012), among which the family Cimicidae (bed bugs) and the subfamily Triatominae (kissing bugs) both being entirely obligate vertebrate blood-feeders (Weirauch et al. 2019).

Opportunistic predators or scavengers could also evolve into micropredators relatively easily. Given the scavenging behavior of hagfishes, the only existent relatives, micropredation of some lampreys might have evolved from scavenging. Among marine invertebrates in particular a shift from scavenging to micropredation is likely, as a number of marine isopods, amphipods, and nudibranchs engage in both scavenging and micropredation. Ranging from obligatory to opportunistically, prey are often sessile or planktonic animals which require little morphological or behavioural adaptation to hunt (Blankenship and Levin 2007; Bloom 1981). This, however, remains to be tested.

An important difference between parasite-host, predator-prey, and micropredator-prey relationships is the levels of co-evolution. Micropredators can usually consume different prey species, indicating low victim specificity, much like a predator. This means relatively few adaptations are required to engage in a micropredatory lifestyle for free-living animals. Even in the case of generalist parasites, we are likely to find longer periods of co-evolution between parasites and their hosts than between predators or micropredators and their prey (Lafferty, 2008). Further, unlike parasitic feeding strategies, which require considerable adaptation to exploit host resources, micropredators should be able to relatively easily shift into predatory or parasitic feeding strategies. Though many micropredators have more specialize feeding habits towards certain taxa (e.g. haematophagous micropredators prefer to feed on mammal or avian prey, due to higher quality nourishment compared with other taxa, like amphibians)(Morishima et al. 2020) than usually observed in predators, even in these cases feeding is seldom physiologically restricted, and they can potentially feed on any species they encounter. For extremely generalist micropredators like leeches, relative size is not even a limiting factor, since small prey can be consumed and killed in a predatory fashion.

The small relative size of a micropredator to its prey is not just beneficial in approaching prey undetected, but also evolutionarily adaptive in lowering the nourishment requirements of their feeding activities and thus reducing the fitness costs on prey. The low fitness costs and little specialization of micropredator feeding, likely causes little evolutionary defense against such attacks to evolve in potential prey species (Lafferty et al, 2015). This is because, many micropredators require significant population densities in order to reduce prey fitness or cause mortality, swarming for example, and is often a seasonally or geographically restricted phenomenon. It is only in species where mortality regularly occurs as a result of micropredator feeding, that we do see defensive behaviours or physiology develop. For example, swarms of biting flies in the summer months on the tundra are more than just a nuisance and cause measurable decreases in caribou fitness as a result of increased time warding off flies and blood loss (Bruce Hunter et al., 1997; Smith et al., 1998). This has led to the behavioural adaptations in caribou herds causing them to temporarily migrate to elevations that flies cannot tolerate in order to rest (Toupin and Manseau 1996).

Ecological Significance of micropredators

Trophically, parasites and free-living consumers have an inverse relationship. That is, with each level upward on a trophic cascade consumer abundance decreases, while parasite abundance increases, tending to gather at their greatest numbers and variety in apex predators (Laffery et al. 2006; 2008). This one of the major ways parasites are believed to influence community structure and regulate food web interactions (Arias-Gonzalex and Morand 2006; Lafferty et al. 2006). Micropredator feeding on the other hand is behaviourally like a predator and impacts its prey's resources like a parasite (Lafferty et al. 2015), but unlike a parasite the resource loss due to micropredator feeding is not constant and is spread out across multiple trophic levels. It is likely, therefore, that micropredators trophically have a low but consistent impact across multiple levels of consumers, without increasing or decreasing in number and diversity. For example, a vampire but will feed on a cow, chicken, dog, or human with little loss for the prey and no wide trophic implications. Herbivores (i.e. micropredators of sessile organisms) are a major exception to this, as they are exclusively primary consumers in all terrestrial food webs and have a direct influence on the number and diversity of producers (Willis and Memmott 2005).

While micropredators themselves have limited impact on trophic level, the parasites they carry do have significant influence on community structure, as a disease will affect specific species disproportionately. Hypothetically, any species can act as a vector for disease, but as a result of their generalist feeding habits and lack of lethality from feeding, all potential prey species of a micropredator will also be exposed to pathogens the micropredator encounters, greatly increasing the number of opportunities novel diseases have to infect new hosts. This can prevent the establishment of species from colonizing environments that would otherwise be tolerable. For example, in sub-Saharan Africa the spread of parasitic disease throughout the continent has resulted in shifts in wild and domestic animal populations as a result of changes in the species of tsetse fly vector and *Trypanosoma* parasite being spread, as some species of the disease are more lethal to some hosts than others (Krafsur, 2003; Roditi and Lehane 2008). The effects of vectors on animal populations have been extensively studied, and I will not go into further detail on them here.

While micropredators do not require the death of their prey to feed, swarming behaviour or relative size of the micropredator to its prey can result in fitness loss and death. Attacks from micropredators can cause significant damage to prey tissue and open wounds cause secondary infections. High micropredator densities also cause behaviour changes in prey, as prey may actively avoid environments entirely or spend longer grooming and moving to reduce micropredator feeding, thus spending less time resting and feeding themselves, reducing fitness (Toupin and Manseau 1996). Swarming can also result in a direct loss of condition from blood loss, tissue damage, and hair/plumage loss in some species, particularly in young individuals which can flee or lack a large enough mass to cope with the micropredator feeding. Chick loss in raptors as a result of black fly swarming (Bruce Hunter et al. 1997; Smith et al. 1998), or moose calf death from tick infestation have been reported for decades (Musante et al. 2007), and particularly in the case of moose this has been a growing issue in southern Canada where ticks are invasive (Leighton et al. 2012). Even without swarming, the increased stress resulting from micropredator feeding can cause fitness or growth reductions over time, and the sores or tissue damage can invite secondary infections, lowered body condition, or reduced chances of finding a mate.

Some groups of micropredators, most notably the biting flies, are often also pollinators, and so are important for reproductive cycle of many plants, in addition to the negative effects they have on prey animal populations (Gorham 1976). Likewise, many of the so called "cleaner" species such as ox-peckers or cleaner wrasses play a key role in removing ectoparasites and dead tissue from larger species, with the impact of cleaner fish in particular being very important to the health of reef fishes (Sato et al. 2020). Yet, these species are also often micropredators, feeding on the living tissue of their prey as well as ectoparasites when the chance presents itself. Among wrasses in particular, the evolution of so called "false-cleaners", which are fin-clipping obligate micropredators that mimic cleaner fish (Fujisawa et al. 2018; Sato et al. 2020), is an example of how micropredation can arise in these symbiotic relationships.

Micropredator contact with prey and dispersal

So far, I reviewed the evolution of micropredation and the ecological significance of micropredators. What is critically lacking is the information on dispersal and spatial structures of micropredators, which significantly affect many ecological and evolutionary processes, such as distribution, colonization, population dynamics, local adaptation, and speciation (Cayuela et al. 2018). Parasites form infracommunities within hosts (i.e. the population of a parasite species occupying an individual host) and their dispersal strongly depends on host dispersal. Thus, spatial structure of parasites often follows that of hosts

(Mazé-Guilmo et al. 2016). Predators, on the other hand, are free-living and their spatial structure depends on their own dispersal ability, as well as environmental tolerance. Micropredators can move either by themselves or by dispersal via prey. Thus, it is generally predicted that dispersal and/or spatial structure of micropredators show intermediate patterns between free-living organisms and parasites when all else being equal (Fig.2-3), although this is currently untested.

Requirement of case studies

During the literature review, I noted that one of the main reasons for the under appreciation of micropredators is the lack of representative case studies. The case studies that do exist focus on micropredator feeding habits and evolution (Jones et al. 2007, 2008), and rarely discuss their population structure and dynamics. Therefore, in this PhD thesis, I provided a case study of the micropredatory leech, *Taimenobdella amurensis*, and their main prey fish, *Salvelinus curilus*, in the Sorachi River, Hokkaido, Japan. Since the metapopulation dynamics and genetics of the prey fish have been studied for more than 20 years, it is ideal for investigating micropredator-prey relationships. Additionally, as a stream dwelling species, these micropredators live in a highly restricted environment, preventing active dispersal of the micropredator between stream populations within the same river due to the unidirectional flow of the current (stream-drift) and narrow width of the stream environment (Blasco-Costa et al. 2012). Thus, these micropredatory leeches should be completely reliant on prey movement to connect populations, a situation that is common for parasites but rare in predators. Because of the close parasite-host co-evolutionary relationship, parasite populations often closely mirror the populations of their most vagile hosts (Blasco-Costa and Poulin 2013), and due to their lack of such relationships, predator-prey populations rarely display such a pattern. As micropredators share traits with both predators and parasites, in a highly restricted environment the micropredatory leeches should be at a large disadvantage in moving between populations, since they lack the parasite's close relationship with its host or the predator's ability to disperse themselves. Under such conditions, I would therefore expect these leeches to form highly structured metapopulations as a result of the low rates of geneflow between stream populations, independent of trends in prey fish movement.

I also examined the generality of micropredator-prey genetic structure by reviewing past literature. Following the protocol of Mazé-Guilmo et al. (2016), I compared the genetic structure of micropredators, herbivores (micropredators of sessile organisms), predators (or free-living organisms), and parasites as reported in 103 separate studies. This protocol takes into account a number of life-history traits that should influence the genetic structure of study species, such as: body size, dispersal mode, host/prey dispersal mode, sexual mode, etc.., and provided a more accurate representation of the pattern. If my predictions are correct, free-living predators should have the largest amount of genetic structuring over distance, while parasites have the least, and micropredators will be situated between predators and parasites.

Tables

Table 2-1 Clades with known examples of predatory, micropredatory, and parasitic species. Clades containing all three are highest priority for review.

Clade	Rank	Predator	Micropredator	Parasite
Amphipoda	Subclass	yes	yes	yes
Aves	Class	yes	yes	no
Coleoptera	Order	yes	no	yes
Copepoda	Subclass	yes	no	yes
Diptera	Order	yes	yes	yes
Hemiptera	Order	yes	yes	no
Hirudinea	Subclass	yes	yes	no
Hymenoptera	Order	yes	no	yes
Isopoda	Subclass	yes	yes	yes
Lepidoptera	Order	yes	yes	yes
Mammalia	Class	yes	yes	no
Mesostigmata	Order	yes	yes	yes
Polychaeta	Class	yes	no	yes
Siphonaptera	Order	no	yes	yes
Teleostei	Infraclass	yes	yes	yes
Gastropoda	Class	yes	yes	yes

Table 2-2 Google Scholar search results by organism and term. Due to high number of results in some categories, only results from 2019 were recorded.

Organism	Year	Parasite	Predator	Micropredator	Vector
Piscicolidae	2019	47	0	0	28
Mosquito	2019	6620	15	28	7840
Tick	2019	4610	1060	26	6460
Flea	2019	983	632	12	1500
Lamprey	2019	485	407	8	735
Blackfly	2019	468	0	0	547
TseTse Fly	2019	1410	0	0	1230
Cookiecutter Shark	2019	0	46	0	0
Vampire Bat	2019	0	27	2	169
Gnathiid Isopod	2019	55	40	18	22
Mesostigmatid Mite	2019	85	91	0	52
Sandfly	2019	2410	190	0	1760
Horse Fly	2019	1640	695	3	1510
Deer Fly	2019	1600	1160	12	752
Midge	2019	1440	493	10	972

Table 2-3 SCOPUS search results by organism and term. Due to low number of results in some categories, all results were recorded.

Organism	Year	Parasite	Predator	Micropredator	Vector
Piscicolidae	all	56	1	0	12
Mosquito	all	9097	883	2	25571
Tick	all	8445	138	1	10637
Flea	all	1733	187	1	1696
Lamprey	all	142	97	0	33
Blackfly	all	170	35	0	261
TseTse Fly	all	1268	15	0	1550
Cookiecutter Shark	all	0	4	0	0
Vampire Bat	all	21	9	0	53
Gnathiid Isopod	all	81	9	8	8
Mesostigmatid Mite	all	38	40	0	12
Sandfly	all	649	3	0	1084
Horse Fly	all	177	5	0	210
Deer Fly	all	103	10	0	71
Midge	all	151	28	1	669

Figures

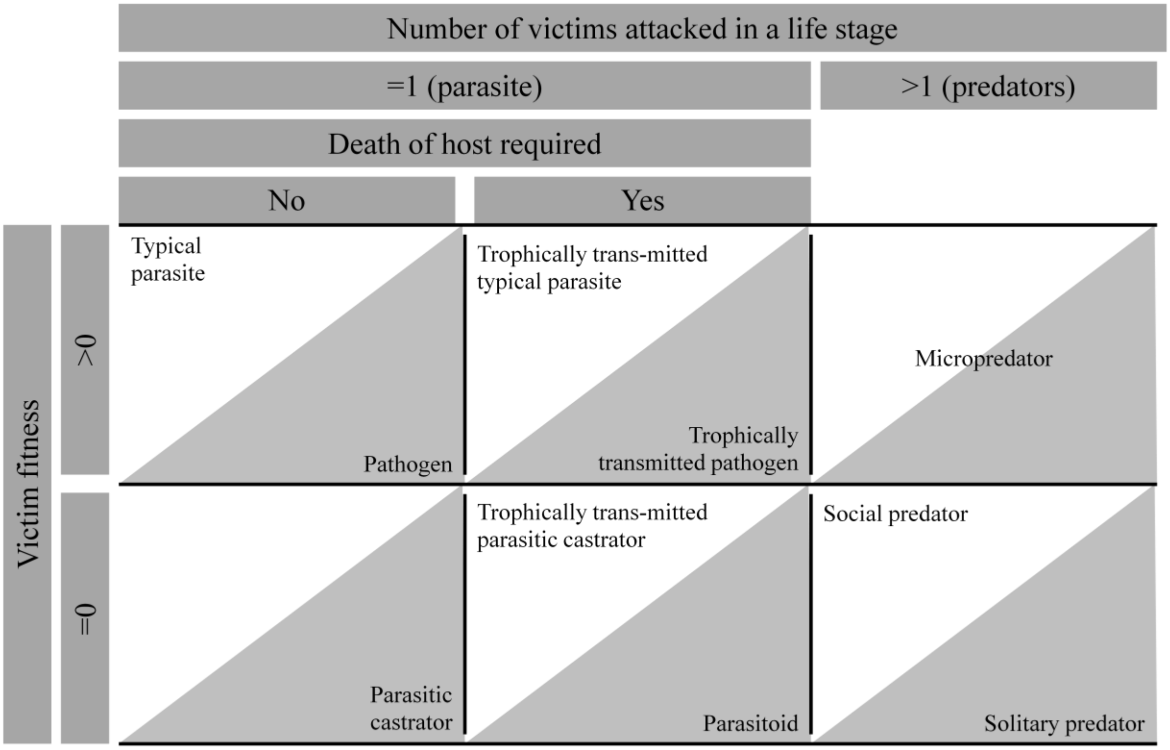

Figure 1 in Lafferty and Kuris (2002) *TREE*

Fig. 2-1 The 10 consumer strategies described in Lafferty and Kuris (2002). Their figure visually represents how the four dichotomies can be applied to separate consumer strategies into categouries. Intensity-dependent and independent pathology is shown by the positioning either above or below the diagonal lines , respectively; note that micropredators are placed centrally indicating both strategies.

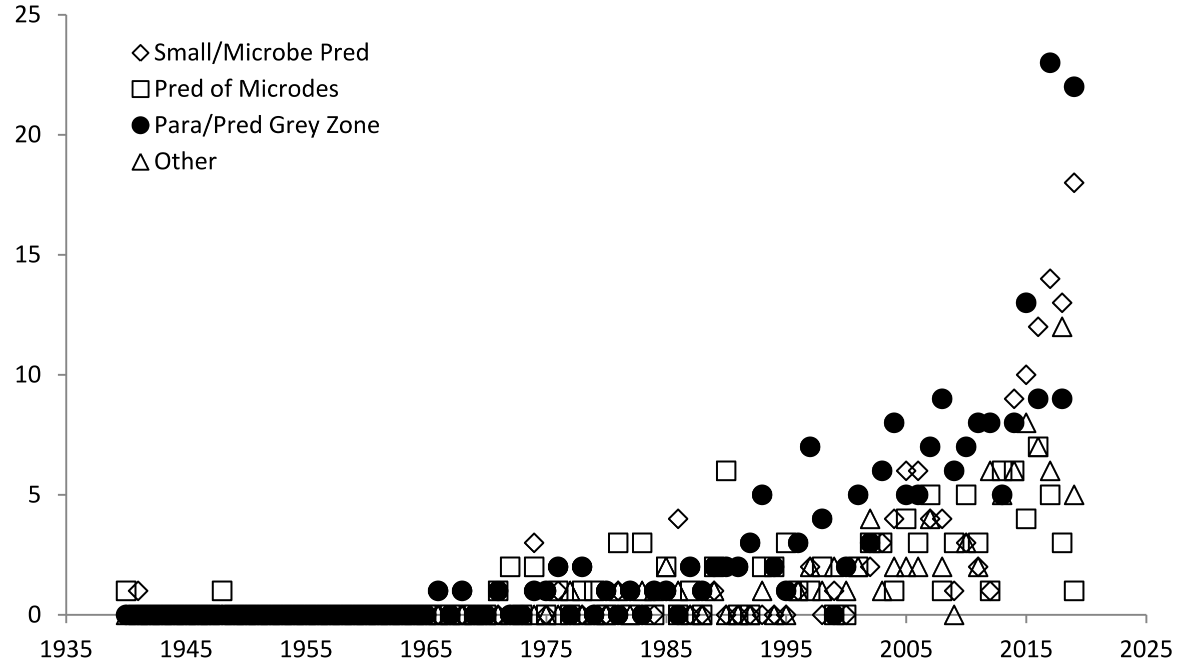

Fig. 2-2 Google Scholar search results for how the term "micropredator" has been used over time. The meaning used in this review has been the most common use of the term, though small predators or predatory microbes, as well as predators of microbes have also recently seen an increase in use.

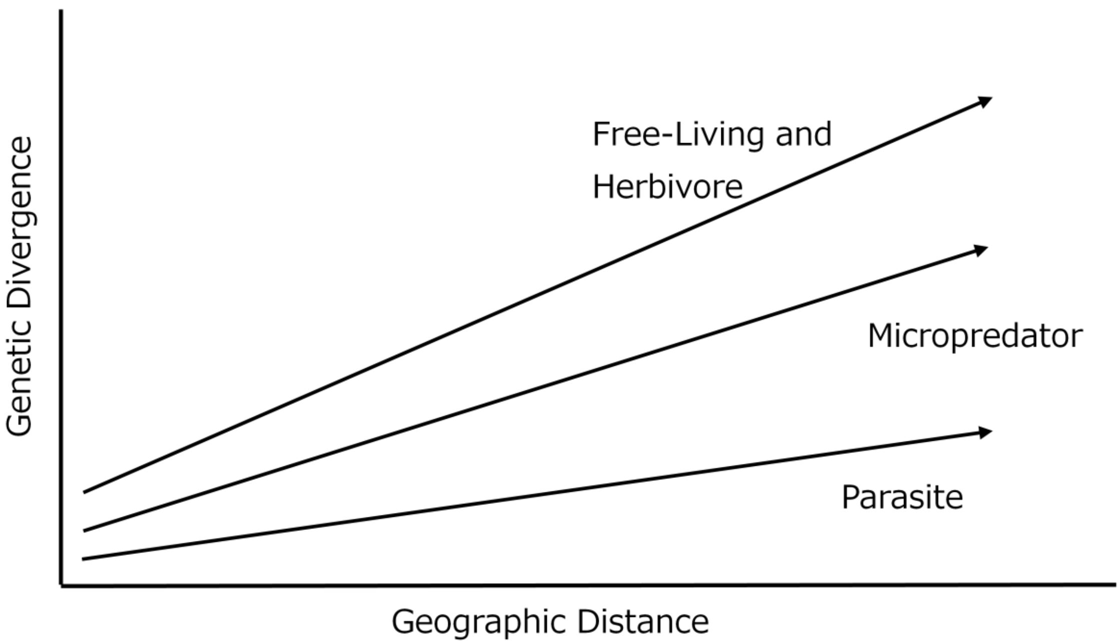

Fig. 2-3 Though micropredators of motile organisms are functionally free-living, they can utilize prey movement to disperse, similar to but less efficient than parasites. As a result with all else being equal, micropredator populations should display more genetic divergence over distance than parasites but less than free-living species, such as predators. On the other hand herbivores are unable to use their prey for passive dispersal and so will be similar to free-living animals.

Chapter 3: Micropredator-prey genetic structures: does the dispersal of a piscivorous stream leech reflect the dispersal of their main prey?

Micropredation is one of the major trophic or consumer strategies, widely observed in different clades of animals, such as biting flies, lampreys, and vampire bats (Chapter 1). Micropredators consume only a small part of their prey and, therefore, do not require the death of the prey, which contrasts with predation. Feeding in some species is characterized by relatively long prey attachment time periods (e.g. days or weeks): which has led some researchers to consider them parasites. Haematophagous animals are the most representative group of micropredators (Chapter 1). They play significant roles in ecosystems, particularly via the spread of pathogens and diseases. Thus, spatial structure and dispersal of micropredators is crucial to understand their ecological roles and evolutionary relationships with their prey, though these have been largely underappreciated (Chapter 1).

There are relatively few studies evaluating the dispersal of micropredators, compared to parasites and predators. This is partly because of the difficulty in tracking their movements and finding prey with micropredators attached. For example, the attachment of sea lampreys to fishes and aquatic mammals have been well reported, but how long they attach to prey and how far they are carried is largely unknown. Genetic analysis can infer the dispersal or gene flow of any organism among different locations. In fact, genetic analysis revealed very weak genetic structuring in sea lampreys over large geographic scales, which suggests that mobile prey aid the wide-spread gene flow of micropredatory lampreys (Waldman et al. 2008; Mateus et al. 2021). Nakano et al. (2020) also found long-distance dispersal of micropredatory leech that attach to the eyes of seabirds. On the other hand, some terrestrial leeches that mostly predate on mammals showed very high local genetic structure, suggesting highly limited dispersal via mammalian movement (Morishima & Aizawa 2019).

Here, I investigated the spatial population structure and gene flow of a micropredatory stream leech, *Taimenobdella amurensis*, in a stream network of the Sorachi River, Japan, by employed newly developed custom microsatellite markers. The leech rely heavily on cold spring-fed streams, where the main prey fish, *Salvelinus curilus,* also inhabit (Katahira et al. 2017). This is an ideal system to uncover the unique characteristics of micropredator-prey relationships, because the metapopulation dynamics and genetics have been intensively studied for the prey fish over the last two decades (e.g. Koizumi and Maekawa 2004; Koizumi et al. 2006, 2008, Koizumi 2011; Katahira et al. 2017). By comparing the genetic structures of the micropredator and prey, we can infer not only the dispersal mode but also the mechanisms of population persistence of the locally distributed micropredators.

MATERIALS AND METHODS

STUDY SITE AND SPECIES

T. amurensis is an endemic species to Northeast Asia, reported from the Amur River, Russia, and rivers in Hokkaido, Japan (Nagasawa et al. 2009; Katahira et al. 2017). Currently little is known about the natural history of the leech, but they seem to have an annual life cycle and low prey specificity, though most cases reported from freshwater salmonids (Nagasawa et al. 2009; Katahira et al. 2017). As an exclusively lotic species, upstream dispersal is only possible while leeches are attached to prey fishes.

Sampling was conducted in multiple tributaries of the Sorachi River, in central Hokkaido, Japan (Fig 3-1). The Sorachi River has a number of small tributaries (>100) along its length, typically ranging in width from 0.5 metres to 2 metres, and a few hundred meters in length. Separated into two types, spring fed and non-spring fed, *T. amurensis* only forms populations in spring fed tributaries and only very occasionally are leeches found in non-spring fed tributaries or the mainstem. This discrepancy is likely related to the difference in water temperature between the two types of tributary with spring fed tributaries maintaining a stable temperature ranging from 5–8°C year-round, while non-spring fed tributaries fluctuate considerable both daily and seasonally (0–16°C)(Koizumi and Maekawa 2004).

Southern Asian Dolly Varden *Salvelinus curilus* are the dominant fish species in this river, particularly in the spring fed and upper reach tributaries. In the Sorachi River, *S. curilus* are landlocked and migrate between the small tributaries where they spawn and form nurseries, and the mainstem which they use for feeding (Koizumi and Maekawa 2004). Non-migratory or resident individuals can also be found in these tributaries, and like many salmonids are almost entirely male, as females tend to migrate to the mainstem by late summer of their first year (Ayer et al. 2017; Koizumi et al. 2006). Other species are often found in the lower reach, mainstem, and non-spring fed tributaries, such as white-spotted charr *S. leucomaenis*, Sakhalin taimen *Parahucho perryi*, freshwater sculpin *Cottus nozawae*, brook lamprey *Lethenteron* sp., and Siberian stone loach *Barbatula toni* (Koizumi et al. 2012).

Because both *T. amurensis* and *S. curilus* strongly depend on spring-fed tributaries, *S. curilus* is the main prey of this leech (Katahira et al. 2017). Leeches have also been observed on white-spotted charr and freshwater sculpin in this river system (Katahira et al.

2017), as well as *P. perryi* and masu salmon *Oncorhynchus masou* in other parts of their range (Nagasawa et al. 2009), confirming generalist feeding habits in this species. While *T. amurensis* are generally rare, their prevalence and intensity vary from tributary to tributary, with anywhere from 0-17% of fish captured and between 1 to 7 leeches attached at a time (Katahira et al. 2017; CG Ayer, unpublished data). Though the full lifecycle of *T. amurensis* remains unknown, it is reasonable to assume based on field observations that they have an annual lifecycle, since a size overlap between seasons has never been observed (Katahira et al. 2017). Leeches have only ever been observed while attached to fish prey, so non-feeding behaviour and cocoon placement are unknown.

FIELD SAMPLING

Leeches were collected in multiple seasons over multiple years, with most being collected in June as part of an annual Dolly Varden census in the Sorachi River, where fish are captured and measured from 25-30 key tributaries (Table 1). As part of a long running faunal census (20+ years) conducted annually during the spring in the Sorachi River (e.g. Koizumi et al. 2008; Koizumi 2011), *T. amurensis* collection was incorporated starting in 2013 along with the standard measurements taken from target Dolly Varden charr. Dolly Varden were caught using a backpack electro-fisher (Smith-Root, Backpack, ElectroFisher

Model 12B-POW), and all captured fish were anesthetized using FA100 (DS Pharma Animal Health Co., Ltd.) before having their fork length (to the nearest millimeter) and a clipping of their adipose fin taken and stored in 70% ethanol for genetic analysis. After fish were measured, they were immediately placed in a recovery net before being released back to the locations they were captured from. Any leeches found on fish or within holding containers were carefully removed with forceps and placed in vials containing 99% ethanol for later DNA extraction. Because *T. amurensis* population densities are unknown, sampling in 2017 and 2021 was limited to roughly 30 individuals per population to prevent oversampling. Leech identification followed the criteria of Furiness et al. (2007) and Nagasawa et al. (2009), and was further confirmed by Katahira et al (2017). According to all existing records, *T. amurensis* is the only species of piscicolid leech found in the Sorachi River.

Microsatellite Primer Development

Since 2013, a total of 1228 leeches have been collected from 17 tributaries in the Shiisorapuchi River. Of these, 685 caught in the spring of 2014, 2016, 2017, and 2021 from 12 tributaries (labeled HO, IKU, KU, PA, SI, SZ, T11, T20, T49, T50, T50.5, TA, and TS) (table 3-1), as well as 8 individuals from a single population (TA) in the summer of 2019, have been genotyped for this study. An additional 24 leeches from the Chitose River were provided by the Chitose Salmon Museum during the Autumn of 2015, for a genetic analysis between watersheds comparing the Chitose River population with the Sorachi River population.

Total DNA was extracted from whole leeches or tissue clippings (2 mm × 5 mm), depending on individual size, using the PureGene DNA isolation kit (Applied Biosystems) according to the manufacturer's instructions. Microsatellite enriched libraries were isolated following Glenn and Schable (2005), and 13 custom microsatellite primers were developed for the population genetic analysis in this experiment (*Eb130*, *Eb142, Eb169, Eb172, Eb187, Eb192, ER107, ER122, ER143, ER146*, *ER171*, *ER177*, and *ER190*). M13 multiplex primers were applied to the custom leech primers and labeled at the 5' end with FAM, NED, PET, VIC fluorescent dyes (ABI, Table. 3-2). Following the protocol of Blacket et al. (2012), Polymerase Chain Reaction (PCR) was conducted on 717 leech samples, 24 from the Chitose River and 693 across various tributaries and years in the Sorachi River. Reaction volumes were 11ul and contained 0.1 ul forward primer, 0.2 ul reverse primer, 0.1 ul labeled M13 multiplex forward primer, 5 ul of Qiagen multiplex mix (Qiagen Type-it Microsatellite PCR Kit), 1 ul extracted DNA, and the remaining 4.6 ul was RNase-free water. The PCR thermocycling protocol for the microsatellite DNA analysis was as follows: 15 min at 95ºC, followed by 40 cycles of denaturation at 94ºC for 30 s, annealing at 59ºC for 90 s, and extension at 72ºC for 60 s, once cycling is complete an additional 30 min at 60ºC was recommended by the Qiagen multiplex mix manufacturer. The amplified products were analyzed on the genetic analyzer ABI 3130 (Applied BioSystems) with GeneScan 500 Liz size standard (Applied BioSystems), and allele sizes were scored using GeneMapper (GeneMapper ver. 4.0; Applied BioSystems).

POPULATION GENETIC ANALYSIS

Tests for Hardy-Weinberg equilibrium (HWE) were performed on the Microsoft Excel program extension Gen AlEx ver. 6.503 (Peakall and Smouse 2006, 2012). Tests for linkage disequilibrium (LD) were performed on Genepop on the web (Rousset 2008) using the Chitose River samples and 7 representative sites from the Sorachi River chosen based on sample size (n>30). A principal coordinate analysis comparing linearized pairwise *Fst* values from sites with multi-year sample data, was performed in GeneAlEx to visualize clustering patterns of different sampling sites and years. To test for statistical population differentiation between sites, an analysis of molecular variance (AMOVA) was also conducted using GeneAlEx. To account for the possibility of null alleles, observed heterozygosity (H_o) and expected heterozygosity (H_e) were evaluated using Inest ver. 2.2

(Chybicki and Burczyk 2009), and measurements of genetic differentiation between populations (*Fst*)(Weir and Cockerham, 1984) were calculated using FreeNa (Chapuis and Estoup, 2007). Spatial genetic structure was determined by testing the significance of Isolation by distance (IBD; Wright 1943) with a Mantel test between a matrix of linearized pairwise *Fst* (*Fst*/1-*Fst*) values calculated using FreeNa (3-3), and a matrix of pairwise geographic site distances (km) within the Sorachi River. Dolly Varden loci data, pairwise *Fst*, linearized pairwise *Fst*, and pairwise site distance calculations were calculated for a previous study, and provided by Koizumi et al (2006).

A STRUCTURE analysis (ver. 2.3.4) was performed to assess population structure, using all 13 microsatellite loci (Pritchard et al, 2000), a user-defined number of clusters *K* was used under the following conditions: 200000 replicates after a burn-in of 100000. STRUCTURE output data was analyzed using STRUCTURE HARVESTER ver. 0.6.94 (Earl and vonHoldt, 2012).

RESULTS

While all 13 primers were successfully amplified, primer amplification results varied considerably depending on site and loci. For the Chitose River samples, 12 were polymorphic and one was monomorphic (*ER190*), whereas all 13 were polymorphic for the Sorachi River samples depending on site, and 4 loci were monomorphic at more than 2 sites (*Eb142*, *ER122*, *ER172*, and *ER190*). For polymorphic loci, the number of alleles ranged from 4 to 11 depending on population, observed heterozygosity ranged from 0.12 to 0.61, while expected heterozygosity ranged from 0.16 to 0.66 (Table. 3-4). Heterozygosity corrected for null alleles was calculated using Inest ver. 2.2 (Chybicki and Burczyk, 2009) and the presence of null alleles was suggested at three loci (*Eb142*, *ER122*, and *ER146*) for Chitose River samples, and four loci (*Eb142*, *Eb192*, *ER107*, and *ER122*) for Sorachi River samples. For Chitose River only one locus (*Eb142*) significantly deviated from HWE following a Bonferroni correction. For the Sorachi River HWE was highly depending on site, with only three loci never deviating for any site or locus (*Eb172*, *ER171*, and *ER190*). Following a Bonferroni correction, deviations from LD were found between two pairs of loci from the Chitose River samples (*Eb142*/*ER122* and *ER122*/*Eb172*), and two pairs of loci from only a single population among the Sorachi River samples (*Eb169*/*ER177* and *Eb169*/*ER107*). Deviations from HWE are likely the result of small populations, as well as type I errors. Many sites in the Shiisorapuchi River are expected to have small effective populations, frequent extinction-reintroduction events, and high levels of inbreeding (Katahira et al. 2017), which should contribute to the number of monomorphic loci across sites. Additionally, many of the populations with more than two loci deviating from HWE, were isolated above a natural barrier, which should further limit gene flow and increase inbreeding.

The principal coordinate analysis found clustering among sites, and displayed relatively little separation between years within the same sites (Fig. 3-2). AMOVA results found significant genetic differentiation between populations (*Fst* = 0.28; $p < 0.01$) when comparing all 27 populations and 13 loci. However, Mantel test results found no significant relationship between linearized pairwise *Fst* and geographic distance (*Rxy* = 0.11; $p = 0.40$), indicating no significant isolation-by-distance (IBD) (Fig. 3-3).

The STRUCTURE analysis for all 717 samples using the 13 microsatellite loci, found the highest ΔK value to be $K = 2$, with two much smaller values at $K = 10$ and $K = 18$ (Fig.3-4b). The probability of the data [LnP(D)] increased rapidly with increasing *K* values, almost reaching a plateau at $K = 11$ (Fig.3-4a). This unusual result is likely due to several small populations and deviations from HWE at several loci in a number of populations suspected of high rates of inbreeding, particularly IKU, TA, and TS. Within population STRUCTURE analyses, and exclusion of several small populations ($N < 15$) are required to understand the population structuring in the Sorachi River.

DISCUSSION

I found extremely high genetic divergence (F_{ST} = 0.1-0.3) even at a very small geographic scale (< 10 km). This is among the highest microsatellite divergence so far reported for natural animal populations (i.e. no artificial disturbance, like fragmentation). No pattern of isolation-by-distance was observed. This strongly suggests that there is no functional gene flow among the tributary populations and the effects of genetic drift have accumulated. The local population sizes of the leech are considered to be very small inferred from the small habitat size (only a few hundred meters of the streams) and small population sizes of the prey (usually less than 100 adult fish)(Koizumi et al. 2008; Koizumi 2011; Katahira et al. 2017). Thus, the micropredatory leech can persist a relatively long time even with small, isolated populations. This is also supported by the temporal stability of genetic structure, although the time span of the sampling was not long (3-7 years). Hermaphrodite mating might contribute to the persistence and genetic diversity of the small local populations, because mating with any individual increases the mating opportunity and combination of genetic admixtures (Jarne 1995; Mazé-Guilmo et al. 2016).

The micropredatory leech can disperse much further if they attach to mobile prey than when reliant on their own vagility. In fact, large adult leeches were observed on the bodies of migratory *S. curilus* during their breeding season in autumn (Katahira et al. 2017).

The leech can survive in the mainstem, the migration corridor, at least temporally (Ayer et al. unpublished data) and therefore I had expected some gene flow among tributary populations. The high divergence (F_{ST} = 0.14) was very surprising between KU and SI, which are separated by only 500m and both micropredator and prey are very abundant (F_{ST} = 0.01 for the prey fish). This result suggests one or some combination of the following mechanisms: (1) contact (attachment) time is very short, (2) prey fish use only a single tributary for spawning (no between-tributary movement during breeding season), or (3) the prey die relatively soon after micropredation due to secondary infections or predation by other animals via behavioral changes caused by the micropredator. So far, very limited gene flow was also inferred from a micropredatory leech that mainly preys on terrestrial mammals (Morishima & Aizawa 2019), while archipelago level long-distance gene flow was suggested in a leech that preys on seabirds (Nakano et al. 2020). Thus, dispersal and population structure of this piscivorous stream dwelling leech is more similar to the terrestrial mammalian leech.

High genetic divergence without isolation-by-distance could also imply extinction-colonization metapopulation dynamics. If dispersal and thus colonization are too rare, the number of colonizers will be only a few individuals, which easily creates very strong genetic drift, or founder effect: in this case no correlation between genetic and geographic distance is expected (Kennedy 2012). Though not common, I occasionally observed leeches from fish caught in the mainstem or in non spring-fed tributaries (Katahira et al. 2017). I even found a seemingly new colonization event in a remnant non spring-fed tributary. I found a few individuals in 2018 and 5-10 individual leeches were observed thereafter (though we did not collect samples for genetic analysis to prevent local extinction). Further monitoring is required to clarify these micropredator-prey metapopulation dynamics.

More than 100 piscicolid leech species have been reported from freshwater and marine environments (Light 2007) and some are reported as vectors (Hamilton et al. 2005; Khan 1991; Wright et al. 1999). Our study suggest that the spread of disease may be locally limited, although different leeches might have different contact time. Some researchers call them parasites, but micropredators have different ecological characteristics from parasites. Further research is needed to identify the general ecological or evolutionary patterns of micropredators. I hope this case study merits future work.

Tables

Table 3-1 Field collection results for genotyped *T. amurensis* samples.

Population	River/Tributary	Year	Collected (*N*)	Genotyped (*N*)
CH	Chitose River	2015	24	24
HO	Horoka Shiisorapuchi	2021	15	8
IKU-2021	Ikutora River	2021	33	32
KU-2014	Kuma no Sawa (Shiisorapuchi)	2014	31	31
KU-2017	Kuma no Sawa (Shiisorapuchi)	2017	38	32
KU-2021	Kuma no Sawa (Shiisorapuchi)	2021	37	32
PA	Panke River	2016	18	18
SI-2014	Sika no Sawa (Shiisorapuchi)	2014	46	32
SI-2017	Sika no Sawa (Shiisorapuchi)	2017	34	32
SI-2021	Sika no Sawa (Shiisorapuchi)	2021	34	32
SZ-2021	Shimizu Sawa (Shiisorapuchi)	2021	42	32
T11-2017	Tributary 11 (Shiisorapuchi)	2017	33	32
T11-2021	Tributary 11 (Shiisorapuchi)	2021	32	32
T20-2014	Tributary 20 (Shiisorapuchi)	2014	31	31
T20-2017	Tributary 20 (Shiisorapuchi)	2017	33	32
T20-2021	Tributary 20 (Shiisorapuchi)	2021	50	32
T49-2016	Tributary 49 (Shiisorapuchi)	2016	20	16
T49-2017	Tributary 49 (Shiisorapuchi)	2017	9	8
T49-2021	Tributary 49 (Shiisorapuchi)	2021	25	24
T50.5	Tributary 50.5 (Shiisorapuchi)	2017 and 2021	7 and 6	13
TA-2014	Taki no Sawa (above waterfall)(Shiisorapuchi)	2014	27	24
TA-2016	Taki no Sawa (above waterfall)(Shiisorapuchi)	2016	62	32
TA-2017	Taki no Sawa (above waterfall)(Shiisorapuchi)	2017	33	32
TA-2019	Taki no Sawa (above waterfall)(Shiisorapuchi)	2019	59	8

TA-2021	Taki no Sawa (above waterfall)(Shiisorapuchi)	2021	47	32
TS-2017	Taki no Sawa (below waterfall)(Shiisorapuchi)	2017	37	32
TS-2021	Taki no Sawa (below waterfall)(Shiisorapuchi)	2021	39	32

Collected (*N*) is total number collected at that site in that year, Genotyped (*N*) is number of individuals genotypes from that site and year.

Table 3-2 Characterization of 13 microsatellite DNA loci tested on 717 individual *T. amurensis*

Locus	**Primer Sequence (5'-3')**	**Repeat Motif**	**Dye**	**T_a (°C)**	**Size Range (bp)**	***A***	**H_o**	**H_e**
Eb130	F: M13-TCGAAGCTCCTCTCACTGGT	(GT)6…(GT)5…(GT)5…(GT)4	NED	59	244-264	9	0.48	0.55
	R: GTTTCTTAGCATCTGCCACATCAACAG							
Eb142	F: M13-ACATGTTTCAGGCTGCCATA	(AC)5	VIC	59	240-260	5	0.12	0.16
	R: GTTTCTTCCAGCAGAAAAAGCGGTTAG							
Eb169	F: M13-GACCTCAGGTTGGATGAGGA	(GT)16	PET	59	208-228	11	0.61	0.66
	R: GTTTCTTCGAAATGCATAGAAAGAACGA							
Eb172	F: M13-AAACACACCTCCATGAAAACG	(AC)4…(AC)8	FAM	59	164-194	5	0.22	0.19
	R: GTTTCTTCGTGTAACGAACACGCCTAC							
Eb187	F: M13-CCTTTGAATGCCGTTTGTTT	(GT)9	FAM	59	198-212	5	0.47	0.54
	R: GTTTCTTCATCCAAATTCCTCTATCGAAAA							
Eb192	F: M13-CAACATCCACACTCGCACAT	(AC)4…(AC)3	NED	59	194-238	5	0.60	0.56
	R: GTTTCTTCTCTGCGCCTCTTTCTTCAG							
ER107	F: M13-GGGGAAAATTCTCTCCAACG	(GT)4…(GT)7	FAM	59	250-272	9	0.54	0.62
	R: GTTTCTTCAAAAATTATCAACGGCTTGC							
ER122	F: M13-GTTCGAGGAGAGATGCGAGT	(TAA)3…(TAA)3…(TAA)4…(TAA)4	PET	59	202-256	6	0.27	0.32
	R: GTTTCTTTGAAACCACGGTTTTACTCC							
ER143	F: M13-CACTGTGGTGGAGGATGTTG	(GT)6…(GT)8	VIC	59	157-177	7	0.41	0.47
	R: GTTTCTTCTTTCACAACACGGGGAACT							
ER146	F: M13-CACCACACTAGCTGCCCTCT	(AC)18	VIC	59	200-254	6	0.54	0.59
	R: GTTTCTTGCACTGCTCCACATAATCCA							

ER171	F: M13-GATGCGAGACAAAACGAGTG	(AG)16	FAM	59	224-242	10	0.60	0.64
	R: GTTTCTTCTGCGTCGTCTTTCAATCAA							
ER177	F: M13-GGGGAACTATGGTGGTTTGA	(AC)10	NED	59	216-252	6	0.57	0.58
	R: GTTTCTTAGACCTTTGTGGAGGGTGTG							
ER190	F: M13-CCTTCTCCTGGTGTCTGTGG	(GT)5…(GT)5	PET	59	164-178	4	0.25	0.27
	R: GTTTCTTAAAAGCCATCATCGACCTGT							

T_a annealing temperature, *A* number of alleles, H_o observed heterozygosity, H_e expected heterozygosity

Table 3-3 Pairwise Matrix of displaying Linear F_{st} values for all sampling sites and years.

CH	HO	IKU-2021	KU-2014	KU-2017	KU-2021	PA	SI-2014	SI-2017	SI-2021	SZ-2021	T11-2017	T11-2021	T20-2014	T20-2017	T20-2021	T49-2016	T49-2017	T49-2021	T50.5	TA-2014	TA-2016	TA-2017	TA-2019	TA-2021	TS-2017	TS-2021	
0.00																											**CH**
0.03	0.00																										**HO**
0.15	0.17	0.00																									**IKU-2021**
0.04	0.10	0.08	0.00																								**KU-2014**
0.07	0.10	0.10	0.00	0.00																							**KU-2017**
0.10	0.11	0.09	-0.01	0.00	0.00																						**KU-2021**
0.10	0.13	0.23	0.11	0.12	0.15	0.00																					**PA**
0.11	0.15	0.28	0.10	0.10	0.14	0.18	0.00																				**SI-2014**
0.20	0.27	0.35	0.18	0.19	0.19	0.30	0.02	0.00																			**SI-2017**
0.21	0.27	0.38	0.18	0.19	0.19	0.31	0.02	-0.01	0.00																		**SI-2021**
0.14	0.18	0.34	0.18	0.15	0.16	0.14	0.21	0.34	0.35	0.00																	**SZ-2021**
0.11	0.11	0.24	0.16	0.14	0.15	0.16	0.17	0.23	0.26	0.24	0.00																**T11-2017**
0.11	0.09	0.22	0.15	0.15	0.17	0.19	0.22	0.29	0.30	0.27	0.00	0.00															**T11-2021**
0.05	0.03	0.13	0.03	0.06	0.06	0.06	0.08	0.13	0.14	0.18	0.06	0.05	0.00														**T20-2014**
0.03	0.05	0.13	0.04	0.04	0.07	0.11	0.05	0.13	0.14	0.17	0.08	0.09	0.00	0.00													**T20-2017**
0.06	0.05	0.12	0.02	0.04	0.03	0.09	0.11	0.15	0.17	0.16	0.09	0.09	0.00	0.02	0.00												**T20-2021**
0.06	0.09	0.18	0.12	0.11	0.13	0.10	0.13	0.27	0.28	0.10	0.15	0.17	0.08	0.06	0.09	0.00											**T49-2016**
0.05	0.06	0.11	0.06	0.05	0.07	0.05	0.13	0.29	0.32	0.11	0.15	0.20	0.05	0.04	0.05	-0.04	0.00										**T49-2017**
0.08	0.10	0.19	0.10	0.12	0.12	0.08	0.13	0.25	0.27	0.11	0.17	0.21	0.10	0.10	0.08	0.00	-0.04	0.00									**T49-2021**
0.07	0.12	0.30	0.15	0.22	0.23	0.24	0.20	0.30	0.31	0.37	0.22	0.19	0.12	0.13	0.18	0.20	0.15	0.21	0.00								**T50.5**
0.10	0.13	0.14	0.08	0.09	0.11	0.12	0.19	0.32	0.31	0.14	0.11	0.07	0.02	0.07	0.07	0.09	0.10	0.11	0.25	0.00							**TA-2014**
0.11	0.11	0.13	0.10	0.11	0.11	0.19	0.19	0.32	0.33	0.16	0.14	0.12	0.06	0.09	0.10	0.13	0.12	0.13	0.28	0.00	0.00						**TA-2016**
0.10	0.12	0.13	0.09	0.08	0.11	0.15	0.15	0.28	0.27	0.15	0.14	0.09	0.03	0.06	0.06	0.10	0.10	0.10	0.27	0.00	0.02	0.00					**TA-2017**
0.10	0.13	0.25	0.15	0.15	0.20	0.30	0.24	0.40	0.40	0.29	0.24	0.20	0.14	0.09	0.15	0.23	0.22	0.24	0.20	0.11	0.11	0.11	0.00				**TA-2019**
0.08	0.09	0.12	0.09	0.10	0.10	0.13	0.17	0.28	0.28	0.19	0.13	0.10	0.06	0.08	0.07	0.12	0.08	0.07	0.23	0.03	0.02	0.01	0.14	0.00			**TA-2021**

0.10	0.11	0.13	0.09	0.12	0.14	0.15	0.21	0.34	0.33	0.14	0.14	0.10	0.06	0.09	0.09	0.12	0.11	0.12	0.28	0.00	0.00	0.01	0.12	0.01	0.00		**TS-2017**
0.13	0.14	0.13	0.11	0.12	0.12	0.18	0.21	0.34	0.33	0.18	0.16	0.11	0.06	0.09	0.08	0.15	0.13	0.13	0.34	0.00	0.01	-0.01	0.15	0.02	0.01	0.00	**TS-2021**

Table 3-4 Summary statistics for all 717 *T. amurensis* samples from 27 sites and years, using 13 microsatellite DNA loci..

Population	**Indices**	**Eb130(N)**	**Eb142(V)**	**ER122(P)**	**ER171(F)**	**Eb169(P)**	**Eb187(F)**	**Eb192(N)**	**ER143(V)**	**Eb172(F)**	**ER190(P)**	**ER146(V)**	**ER177(N)**	**ER107(F)**	**Mean**
CH	*N*	20.00	23.00	19.00	23.00	24.00	17.00	21.00	23.00	22.00	24.00	24.00	24.00	24.00	22.15
	A	9.00	2.00	4.00	10.00	11.00	4.00	2.00	7.00	2.00	1.00	5.00	4.00	7.00	5.23
	Ae	5.80	1.63	2.19	5.26	5.59	2.62	1.57	2.94	1.42	1.00	3.72	3.59	3.77	3.16
	Ho	0.86	0.55	0.62	0.78	0.82	0.64	0.50	0.72	0.45	0.00	0.70	0.81	0.66	0.62
	He	0.84	0.58	0.69	0.84	0.84	0.66	0.47	0.72	0.42	0.00	0.77	0.72	0.78	0.64
	HWE	0.35	**0.00**	0.00	0.95	0.69	0.63	0.82	0.13	0.30	mono	0.01	0.78	0.27	0.41
HO	*N*	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	7.00	7.00	7.00	7.00	8.00	7.69
	A	6.00	1.00	1.00	6.00	3.00	3.00	2.00	3.00	2.00	2.00	4.00	4.00	7.00	3.38
	Ae	3.77	1.00	1.00	4.41	2.25	1.91	1.28	2.61	2.00	1.51	2.80	3.16	5.33	2.54
	Ho	0.86	0.55	0.62	0.78	0.82	0.64	0.50	0.72	0.45	0.00	0.70	0.81	0.66	0.62
	He	0.84	0.58	0.69	0.84	0.84	0.66	0.47	0.72	0.42	0.00	0.77	0.72	0.78	0.64
	HWE	0.32	mono	mono	0.21	0.05	0.02	0.69	0.15	0.01	0.47	0.22	0.47	0.93	0.32
IKU-2021	*N*	5.00	29.00	27.00	32.00	32.00	32.00	17.00	27.00	29.00	32.00	20.00	32.00	32.00	26.62
	A	3.00	1.00	2.00	3.00	4.00	2.00	2.00	1.00	2.00	2.00	3.00	4.00	6.00	2.69
	Ae	2.38	1.00	1.04	1.65	1.93	1.03	1.71	1.00	1.15	1.03	2.01	2.46	3.20	1.66
	Ho	0.26	0.00	0.31	0.47	0.45	0.10	0.47	0.00	0.30	0.10	0.50	0.62	0.58	0.32
	He	0.48	0.00	0.36	0.44	0.54	0.13	0.48	0.00	0.34	0.13	0.60	0.61	0.73	0.37
	HWE	0.16	mono	0.92	0.75	0.00	0.93	0.58	mono	0.69	0.93	**0.00**	**0.00**	0.00	0.45
KU-2014	*N*	0.00	2.00	1.00	25.00	23.00	3.00	11.00	6.00	23.00	31.00	26.00	29.00	17.00	15.15
	A	0.00	1.00	1.00	4.00	5.00	2.00	2.00	4.00	1.00	2.00	3.00	4.00	4.00	2.54
	Ae	0.00	1.00	1.00	3.00	2.57	2.00	1.42	2.06	1.00	1.03	2.41	2.95	2.44	1.76
	Ho	0.00	0.00	0.00	0.56	0.35	0.33	0.00	0.33	0.00	0.03	0.46	0.69	0.18	0.23
	He	0.00	0.00	0.00	0.67	0.61	0.50	0.30	0.51	0.00	0.03	0.59	0.66	0.59	0.34
	HWE	NA	mono	mono	0.20	0.15	0.56	0.00	0.38	mono	0.93	0.16	0.13	**0.00**	0.28

KU-2017	*N*	23.00	31.00	31.00	32.00	31.00	30.00	30.00	30.00	32.00	32.00	32.00	32.00	32.00	30.62
	A	8.00	2.00	3.00	8.00	5.00	3.00	5.00	5.00	2.00	2.00	3.00	5.00	6.00	4.38
	Ae	2.67	1.21	1.40	2.66	2.45	1.65	2.15	2.08	1.24	1.03	1.92	2.99	3.28	2.06
	Ho	0.52	0.40	0.45	0.68	0.63	0.47	0.59	0.59	0.26	0.10	0.48	0.71	0.67	0.50
	He	0.72	0.44	0.49	0.68	0.64	0.48	0.67	0.59	0.25	0.13	0.52	0.68	0.75	0.54
	HWE	**0.00**	**0.00**	**0.00**	0.49	0.00	0.94	0.12	0.84	0.49	0.93	0.53	0.90	**0.00**	0.40
KU-2021	*N*	4.00	10.00	15.00	24.00	32.00	30.00	9.00	4.00	20.00	27.00	32.00	32.00	32.00	20.85
	A	1.00	1.00	2.00	5.00	4.00	3.00	2.00	3.00	1.00	2.00	4.00	4.00	5.00	2.85
	Ae	1.00	1.00	1.07	3.03	2.02	1.60	1.91	1.68	1.00	1.08	1.99	2.71	3.03	1.78
	Ho	0.00	0.00	0.30	0.66	0.52	0.51	0.44	0.40	0.00	0.31	0.58	0.74	0.68	0.40
	He	0.00	0.00	0.35	0.71	0.56	0.54	0.52	0.50	0.00	0.34	0.54	0.65	0.75	0.42
	HWE	mono	mono	0.89	0.44	0.01	0.00	0.37	0.93	mono	0.84	0.57	0.79	**0.00**	0.49
PA	*N*	13.00	15.00	13.00	15.00	7.00	7.00	13.00	16.00	18.00	18.00	7.00	14.00	7.00	12.54
	A	3.00	1.00	2.00	3.00	2.00	2.00	2.00	3.00	1.00	2.00	2.00	6.00	4.00	2.54
	Ae	2.47	1.00	1.08	2.87	1.32	1.51	1.99	1.55	1.00	1.46	2.00	3.63	2.13	1.85
	Ho	0.91	0.00	0.36	0.69	0.43	0.43	0.59	0.54	0.00	0.46	0.47	0.69	0.50	0.47
	He	0.61	0.00	0.40	0.68	0.45	0.51	0.57	0.59	0.00	0.40	0.54	0.74	0.65	0.47
	HWE	0.07	mono	0.89	0.48	0.66	0.13	0.80	0.00	mono	0.31	0.06	0.00	0.25	0.33
SI-2014	*N*	8.00	7.00	11.00	18.00	27.00	11.00	8.00	6.00	16.00	23.00	19.00	23.00	24.00	15.46
	A	4.00	1.00	2.00	4.00	6.00	3.00	4.00	4.00	2.00	2.00	4.00	4.00	4.00	3.38
	Ae	2.29	1.00	1.20	1.82	2.64	1.45	3.28	3.43	1.06	1.87	1.47	2.90	1.30	1.98
	Ho	0.36	0.00	0.32	0.47	0.58	0.38	0.34	0.33	0.38	0.52	0.43	0.56	0.43	0.39
	He	0.60	0.00	0.42	0.64	0.75	0.57	0.57	0.64	0.48	0.63	0.59	0.73	0.58	0.55
	HWE	0.01	mono	0.00	0.09	0.00	0.91	0.01	0.23	0.90	0.10	0.00	0.06	**0.00**	0.19
SI-2017	*N*	26.00	29.00	5.00	32.00	32.00	32.00	17.00	26.00	32.00	32.00	32.00	32.00	32.00	27.62
	A	2.00	1.00	1.00	4.00	6.00	2.00	3.00	4.00	1.00	2.00	4.00	3.00	2.00	2.69
	Ae	1.65	1.00	1.00	1.48	2.66	1.13	1.96	1.50	1.00	1.68	1.42	2.50	1.36	1.56
	Ho	0.55	0.00	0.00	0.35	0.57	0.16	0.47	0.48	0.00	0.37	0.31	0.64	0.28	0.32

	He	0.41	0.00	0.00	0.39	0.66	0.17	0.57	0.56	0.00	0.43	0.37	0.61	0.30	0.35
	HWE	0.06	mono	mono	0.24	0.38	0.71	0.02	**0.00**	mono	0.20	1.00	0.25	0.77	0.36
SI-2021	*N*	25.00	23.00	24.00	31.00	31.00	31.00	19.00	16.00	31.00	31.00	31.00	31.00	31.00	27.31
	A	3.00	1.00	2.00	5.00	5.00	2.00	3.00	5.00	1.00	2.00	4.00	3.00	4.00	3.08
	Ae	1.08	1.00	1.09	2.11	2.05	1.07	1.46	1.71	1.00	1.66	1.62	2.46	1.59	1.53
	Ho	0.45	0.00	0.49	0.59	0.61	0.26	0.50	0.50	0.00	0.51	0.51	0.78	0.52	0.44
	He	0.49	0.00	0.51	0.62	0.60	0.28	0.56	0.61	0.00	0.51	0.51	0.62	0.49	0.45
	HWE	1.00	mono	**0.00**	0.84	0.81	0.85	**0.00**	0.04	mono	0.55	0.02	0.19	0.85	0.47
SZ-2021	*N*	25.00	31.00	31.00	32.00	32.00	28.00	32.00	32.00	32.00	32.00	32.00	32.00	32.00	31.00
	A	3.00	1.00	1.00	5.00	6.00	3.00	2.00	3.00	1.00	1.00	3.00	2.00	2.00	2.54
	Ae	2.33	1.00	1.00	3.05	1.85	2.04	1.72	1.77	1.00	1.00	1.56	1.24	1.91	1.65
	Ho	0.57	0.00	0.00	0.79	0.49	0.63	0.60	0.47	0.00	0.00	0.45	0.23	0.60	0.37
	He	0.63	0.00	0.00	0.68	0.53	0.67	0.42	0.46	0.00	0.00	0.39	0.23	0.48	0.35
	HWE	0.08	mono	mono	0.80	0.80	0.00	0.02	0.19	mono	mono	0.47	0.49	0.16	0.33
T11-2017	*N*	10.00	19.00	10.00	19.00	30.00	19.00	17.00	19.00	17.00	32.00	30.00	31.00	30.00	21.77
	A	4.00	1.00	2.00	3.00	5.00	5.00	3.00	3.00	1.00	2.00	3.00	3.00	4.00	3.00
	Ae	2.56	1.00	1.47	1.45	2.85	3.03	3.00	2.88	1.00	1.95	1.11	1.34	2.42	2.00
	Ho	0.42	0.00	0.33	0.29	0.63	0.58	0.74	0.81	0.00	0.55	0.22	0.32	0.56	0.42
	He	0.66	0.00	0.45	0.42	0.69	0.72	0.66	0.65	0.00	0.50	0.27	0.36	0.64	0.46
	HWE	0.03	mono	0.24	0.08	0.95	0.54	0.73	0.09	mono	0.61	0.99	0.06	0.24	0.42
T11-2021	*N*	0.00	9.00	8.00	15.00	30.00	10.00	3.00	5.00	10.00	26.00	32.00	32.00	31.00	16.23
	A	0.00	1.00	2.00	3.00	6.00	4.00	3.00	3.00	1.00	2.00	4.00	3.00	4.00	2.77
	Ae	0.00	1.00	1.97	1.52	2.50	3.70	2.00	2.63	1.00	1.50	1.21	1.33	2.23	1.74
	Ho	0.00	0.00	0.45	0.46	0.64	0.58	0.32	0.51	0.00	0.50	0.26	0.34	0.61	0.36
	He	0.00	0.00	0.53	0.54	0.66	0.71	0.53	0.60	0.00	0.54	0.31	0.34	0.66	0.42
	HWE	NA	mono	0.50	0.01	0.19	0.31	0.11	0.14	mono	0.03	1.00	0.84	**0.00**	0.31
T20-2014	*N*	1.00	6.00	10.00	22.00	27.00	9.00	12.00	13.00	18.00	29.00	28.00	30.00	24.00	17.62

	A	2.00	1.00	1.00	5.00	6.00	2.00	3.00	4.00	1.00	2.00	5.00	3.00	5.00	3.08
	Ae	2.00	1.00	1.00	3.46	2.98	1.91	2.67	1.62	1.00	1.53	3.03	2.87	1.90	2.07
	Ho	1.00	0.00	0.00	0.50	0.52	0.11	0.42	0.46	0.00	0.38	0.50	0.63	0.33	0.37
	He	0.50	0.00	0.00	0.71	0.66	0.48	0.63	0.38	0.00	0.35	0.67	0.65	0.47	0.42
	HWE	0.32	mono	mono	0.00	0.46	0.02	0.39	0.98	mono	0.63	0.33	0.73	0.00	0.39
T20-2017	*N*	12.00	14.00	13.00	22.00	28.00	16.00	16.00	24.00	15.00	26.00	32.00	31.00	27.00	21.23
	A	9.00	2.00	4.00	5.00	9.00	3.00	5.00	4.00	2.00	2.00	4.00	3.00	4.00	4.31
	Ae	6.26	1.51	1.78	3.98	3.65	1.68	3.37	1.41	1.22	1.87	3.22	2.91	1.90	2.67
	Ho	0.67	0.44	0.40	0.81	0.68	0.46	0.63	0.38	0.32	0.50	0.61	0.69	0.48	0.54
	He	0.85	0.49	0.58	0.75	0.78	0.52	0.74	0.42	0.35	0.52	0.73	0.67	0.59	0.61
	HWE	0.08	0.00	0.00	0.79	0.05	0.30	0.01	0.99	0.67	0.66	0.02	0.28	0.00	0.29
T20-2021	*N*	24.00	29.00	29.00	17.00	32.00	31.00	28.00	29.00	29.00	29.00	32.00	32.00	32.00	28.69
	A	3.00	2.00	2.00	5.00	8.00	4.00	3.00	4.00	1.00	2.00	6.00	4.00	6.00	3.85
	Ae	1.74	1.07	1.07	3.04	4.03	1.85	2.18	1.49	1.00	1.53	3.84	2.59	2.51	2.15
	Ho	0.52	0.38	0.33	0.63	0.73	0.53	0.77	0.48	0.00	0.49	0.67	0.62	0.59	0.52
	He	0.60	0.42	0.36	0.72	0.78	0.61	0.57	0.57	0.00	0.51	0.76	0.65	0.69	0.56
	HWE	0.17	**0.00**	0.85	0.81	0.94	0.00	0.03	0.07	mono	0.56	0.42	0.26	**0.00**	0.34
T49-2016	*N*	14.00	15.00	15.00	15.00	16.00	16.00	14.00	15.00	15.00	15.00	16.00	16.00	16.00	15.23
	A	7.00	2.00	3.00	6.00	6.00	4.00	4.00	3.00	1.00	4.00	5.00	2.00	4.00	3.92
	Ae	5.03	1.14	1.32	3.44	3.91	2.47	2.25	1.74	1.00	1.77	4.03	1.36	1.39	2.37
	Ho	0.76	0.31	0.37	0.80	0.73	0.66	0.84	0.51	0.00	0.51	0.89	0.36	0.32	0.54
	He	0.81	0.37	0.45	0.74	0.76	0.63	0.61	0.53	0.00	0.56	0.75	0.33	0.44	0.54
	HWE	0.14	**0.00**	0.00	0.30	0.57	0.50	0.21	0.32	mono	0.01	0.62	0.46	0.01	0.26
T49-2017	*N*	2.00	2.00	2.00	2.00	7.00	7.00	2.00	2.00	2.00	2.00	7.00	7.00	7.00	3.92
	A	4.00	1.00	1.00	3.00	4.00	3.00	2.00	2.00	1.00	1.00	5.00	3.00	4.00	2.62
	Ae	4.00	1.00	1.00	2.67	3.38	1.78	1.60	1.60	1.00	1.00	4.26	2.00	2.39	2.13
	Ho	0.70	0.00	0.00	0.67	0.67	0.49	0.43	0.42	0.00	0.00	0.83	0.49	0.54	0.40
	He	0.69	0.00	0.00	0.62	0.72	0.58	0.49	0.49	0.00	0.00	0.76	0.63	0.68	0.44

	HWE	0.42	mono	mono	0.57	0.40	0.48	0.64	0.64	mono	mono	0.35	0.07	0.28	0.43
T49-2021	*N*	19.00	21.00	21.00	21.00	24.00	24.00	21.00	21.00	21.00	21.00	24.00	24.00	24.00	22.00
	A	5.00	1.00	2.00	5.00	7.00	3.00	4.00	4.00	1.00	2.00	5.00	3.00	5.00	3.62
	Ae	3.27	1.00	1.05	2.59	4.78	2.25	2.29	1.64	1.00	1.10	3.65	2.61	1.96	2.24
	Ho	0.78	0.00	0.25	0.65	0.74	0.61	0.89	0.45	0.00	0.26	0.64	0.52	0.43	0.48
	He	0.71	0.00	0.29	0.66	0.80	0.58	0.59	0.51	0.00	0.30	0.74	0.65	0.59	0.49
	HWE	0.65	mono	0.91	0.54	0.04	0.87	0.05	0.98	mono	0.82	0.07	0.05	0.01	0.45
T50.5	*N*	10.00	8.00	8.00	8.00	5.00	5.00	8.00	3.00	6.00	2.00	6.00	9.00	6.00	6.46
	A	4.00	5.00	6.00	6.00	3.00	3.00	4.00	2.00	5.00	1.00	4.00	5.00	4.00	4.00
	Ae	2.33	4.00	4.92	3.28	2.78	2.63	3.05	1.80	3.79	1.00	2.06	3.52	3.43	2.97
	Ho	0.51	0.49	0.62	0.49	0.53	0.35	0.48	0.25	0.49	0.00	0.35	0.50	0.42	0.42
	He	0.70	0.68	0.80	0.75	0.67	0.63	0.70	0.44	0.74	0.00	0.64	0.76	0.70	0.63
	HWE	0.00	0.00	0.27	0.07	0.37	0.38	0.13	0.08	0.56	mono	0.01	0.00	0.16	0.17
TA-2014	*N*	2.00	5.00	6.00	17.00	22.00	4.00	9.00	8.00	19.00	22.00	22.00	24.00	19.00	13.77
	A	2.00	1.00	1.00	3.00	5.00	3.00	3.00	2.00	1.00	2.00	3.00	3.00	2.00	2.38
	Ae	2.00	1.00	1.00	2.64	1.80	2.91	2.22	1.13	1.00	1.20	2.05	1.94	1.87	1.75
	Ho	0.18	0.00	0.00	0.59	0.48	0.38	0.65	0.22	0.00	0.24	0.53	0.60	0.55	0.34
	He	0.42	0.00	0.00	0.64	0.53	0.59	0.58	0.29	0.00	0.25	0.54	0.51	0.48	0.37
	HWE	0.16	mono	mono	0.87	**0.00**	0.22	0.61	0.85	mono	0.64	0.94	0.65	0.57	0.55
TA-2016	*N*	23.00	31.00	30.00	31.00	32.00	32.00	31.00	32.00	30.00	31.00	30.00	32.00	32.00	30.54
	A	5.00	1.00	1.00	3.00	6.00	4.00	3.00	2.00	3.00	2.00	3.00	2.00	4.00	3.00
	Ae	2.59	1.00	1.00	1.70	1.98	1.95	2.20	1.06	1.91	1.07	1.80	1.82	2.43	1.73
	Ho	0.61	0.00	0.00	0.53	0.55	0.57	1.00	0.10	0.74	0.18	0.59	0.63	0.58	0.47
	He	0.70	0.00	0.00	0.45	0.56	0.52	0.55	0.12	0.50	0.20	0.62	0.45	0.61	0.41
	HWE	0.04	mono	mono	0.29	0.04	0.84	**0.00**	0.86	0.02	0.85	0.00	0.03	0.20	0.29
TA-2017	*N*	8.00	12.00	6.00	27.00	32.00	21.00	15.00	14.00	27.00	32.00	29.00	32.00	32.00	22.08
	A	1.00	1.00	1.00	3.00	6.00	4.00	3.00	3.00	1.00	2.00	5.00	5.00	4.00	3.00

	Ae	1.00	1.00	1.00	2.50	2.67	1.56	2.07	1.34	1.00	1.06	2.31	2.01	1.70	1.63
	Ho	0.00	0.00	0.00	0.57	0.63	0.48	0.59	0.36	0.00	0.14	0.60	0.57	0.43	0.34
	He	0.00	0.00	0.00	0.65	0.67	0.58	0.57	0.48	0.00	0.17	0.68	0.55	0.53	0.38
	HWE	mono	mono	mono	0.30	0.03	**0.00**	0.76	0.14	mono	0.86	**0.00**	**0.00**	**0.00**	0.23
TA-2019	*N*	8.00	8.00	8.00	8.00	8.00	7.00	7.00	8.00	8.00	8.00	8.00	8.00	8.00	7.85
	A	4.00	2.00	4.00	4.00	4.00	3.00	3.00	3.00	2.00	1.00	3.00	2.00	3.00	2.92
	Ae	1.71	1.60	3.12	2.91	2.25	1.34	1.34	1.91	1.97	1.00	1.29	1.60	2.13	1.86
	Ho	0.33	0.30	0.89	0.52	0.77	0.43	0.42	0.48	0.88	0.00	0.32	0.36	0.56	0.48
	He	0.57	0.47	0.68	0.69	0.61	0.49	0.49	0.55	0.49	0.00	0.40	0.44	0.57	0.50
	HWE	0.01	0.01	0.01	0.11	0.82	0.98	0.98	0.04	0.03	mono	0.98	0.35	0.64	0.41
TA-2021	*N*	19.00	29.00	29.00	29.00	32.00	32.00	25.00	28.00	29.00	30.00	32.00	32.00	32.00	29.08
	A	5.00	1.00	1.00	3.00	9.00	4.00	3.00	3.00	2.00	3.00	5.00	4.00	9.00	4.00
	Ae	1.67	1.00	1.00	2.39	2.77	1.73	2.16	1.08	1.62	1.23	2.56	2.68	4.10	2.00
	Ho	0.49	0.00	0.00	0.65	0.58	0.44	0.76	0.28	0.54	0.30	0.64	0.53	0.68	0.45
	He	0.52	0.00	0.00	0.61	0.72	0.51	0.56	0.33	0.43	0.33	0.70	0.69	0.80	0.48
	HWE	1.00	mono	mono	0.88	0.01	**0.00**	0.06	1.00	0.06	0.95	**0.00**	**0.00**	0.08	0.37
TS-2017	*N*	28.00	32.00	32.00	32.00	32.00	15.00	32.00	32.00	32.00	32.00	32.00	32.00	32.00	30.38
	A	2.00	1.00	1.00	3.00	6.00	4.00	3.00	2.00	2.00	2.00	4.00	2.00	4.00	2.77
	Ae	1.51	1.00	1.00	2.08	1.92	2.36	2.12	1.17	1.79	1.06	2.77	1.79	2.61	1.78
	Ho	0.57	0.00	0.00	0.55	0.54	0.48	0.97	0.19	0.66	0.11	0.69	0.55	0.65	0.46
	He	0.59	0.00	0.00	0.54	0.54	0.57	0.54	0.20	0.45	0.13	0.71	0.45	0.64	0.41
	HWE	0.00	mono	mono	0.87	1.00	0.00	**0.00**	0.63	0.01	0.86	**0.00**	0.25	**0.00**	0.33
TS-2021	*N*	30.00	32.00	32.00	32.00	26.00	18.00	32.00	32.00	32.00	32.00	24.00	27.00	27.00	28.92
	A	2.00	1.00	1.00	3.00	7.00	4.00	3.00	2.00	1.00	2.00	3.00	4.00	5.00	2.92
	Ae	1.03	1.00	1.00	2.18	1.86	1.80	2.02	1.06	1.00	1.10	1.61	2.00	2.42	1.54
	Ho	0.21	0.00	0.00	0.56	0.50	0.51	0.79	0.12	0.00	0.15	0.42	0.55	0.66	0.34
	He	0.23	0.00	0.00	0.57	0.57	0.53	0.52	0.14	0.00	0.16	0.47	0.61	0.71	0.35

	HWE	0.93	mono	mono	0.01	0.18	0.83	0.01	0.86	mono	0.78	0.30	**0.00**	**0.00**	0.39
All Pop	*Fis*	0.13	1.00	0.21	0.09	0.03	0.27	-0.03	0.16	-0.40	0.05	0.25	0.09	0.27	0.16
	Fst	0.42	0.51	0.46	0.26	0.24	0.17	0.24	0.41	0.35	0.44	0.28	0.21	0.21	0.32

N number of amplified samples, *A* number of alleles, A_e effective alleles, H_o observed heterozygosity, H_e expected heterozygosity, HWE Hardy-Weinberg equilibrium (bold values indicate significant deviation following a Bonferroni correction).

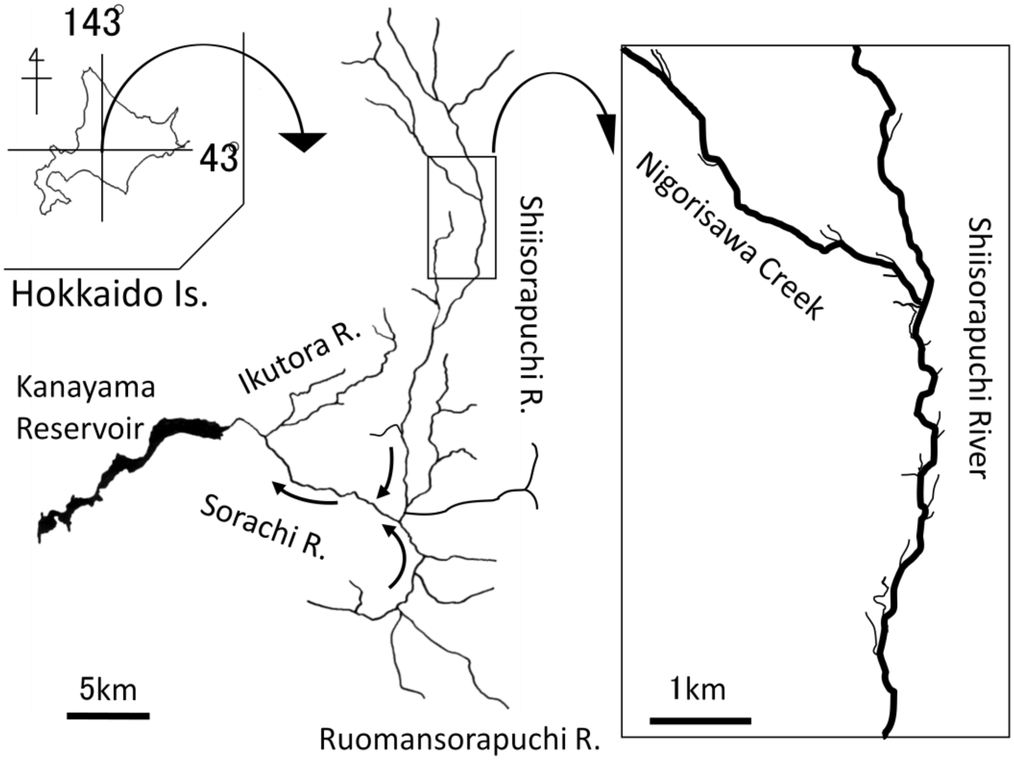

Fig. 3-1 Sorachi River and surrounding major branches

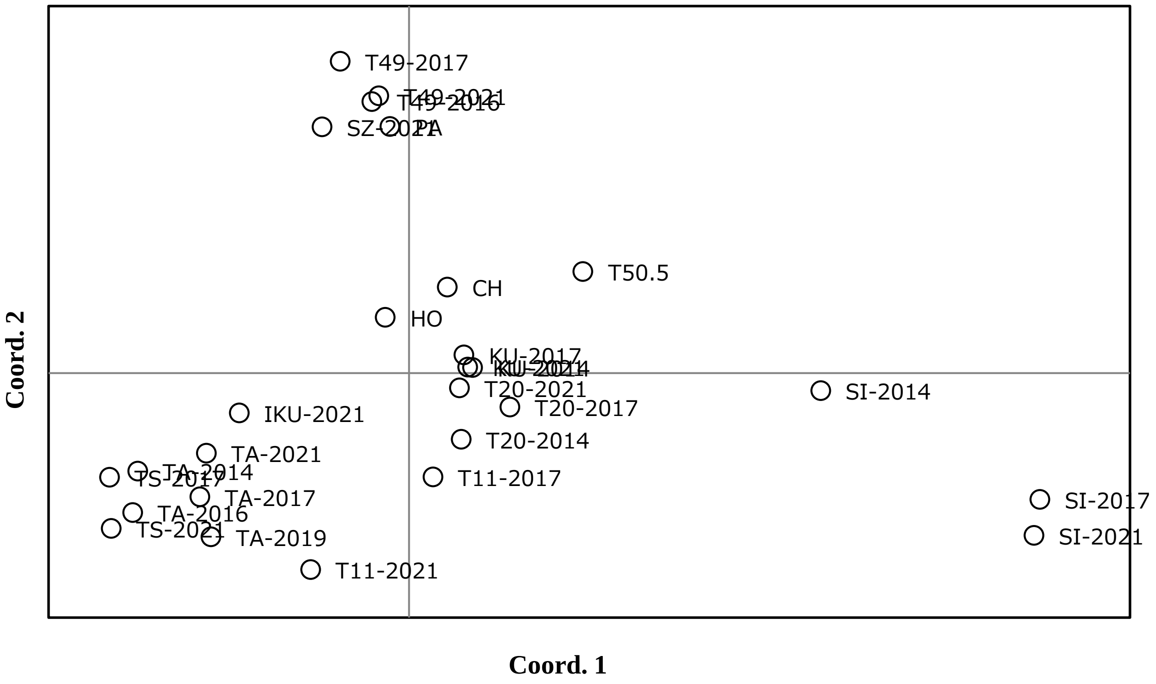

Fig. 3-2 Principal coordinate analysis of *T. amurensis* pairwise linearized F_{st}, clustering of years from the same site indicating relatively little temporal change. Though some geographically close sites appear genetically distant, such as KU and SI which are only 500 meters apart.

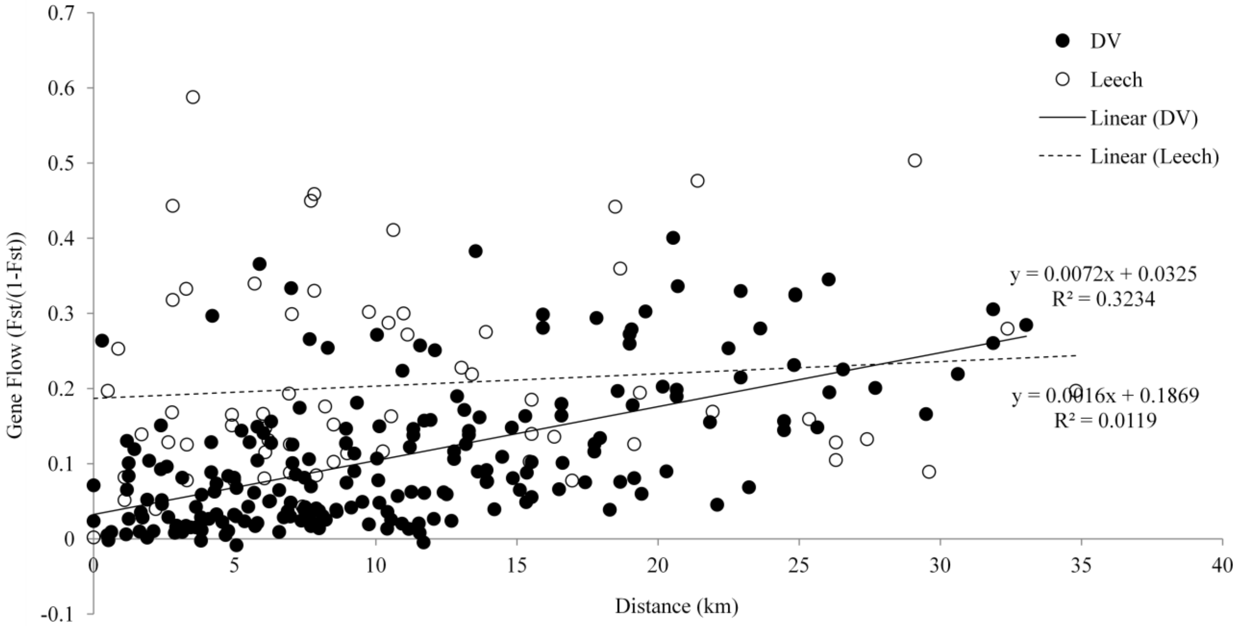

Fig. 3-3 While a significant IBD was found for DV (*R* = 0.57, *p* < 0.01), there was no significant IBD among the leech populations (*R* = 0.11, *p* = 0.40), instead maintaining high levels of population structuring even over short distances.

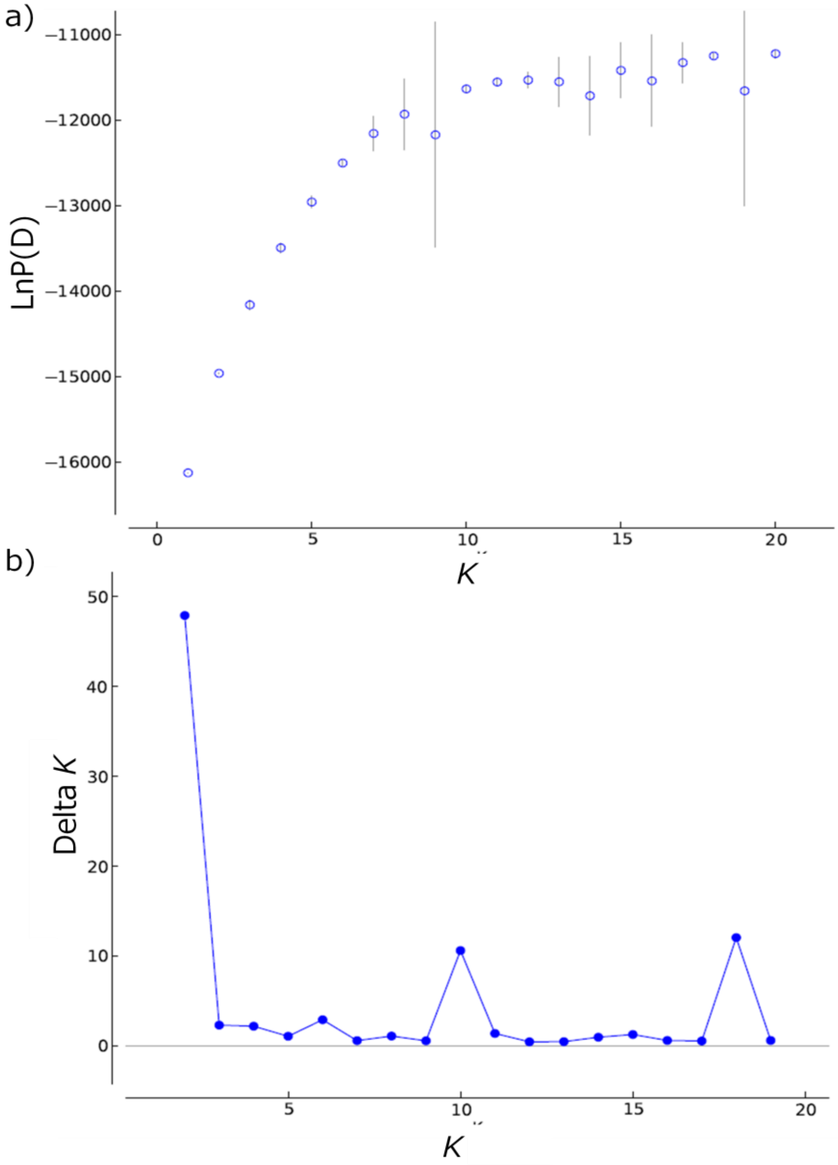

Fig. 3-4 a) Changes in the mean log likelihoods of the data, LnP(D) and rate of change, b) ΔK between successive K values from STRUCTURE analysis of *T. amurensis*.

Chapter 4: General discussion

LITERATURE REVIEW ON DISPERSAL OF MICROPREDATORS

In chapter 3, I found extremely limited gene flow of the micropredatory leech, even with their parasite-like dispersal potential by attaching to a mobile host. Passive dispersal by prey or hosts should depend on the contact time and the contact time is longest in parasites, shortest in predators, and intermediate in micropredators. Thus, I hypothesized that gene flow among local populations is higher in parasites, followed by micropredators, and then predators or free-living organisms, if all else being equal (Fig. 2-3). I also hypothesized that herbivores, which are micropredators on sessile prey, show similar pattern to predators or free-living organisms, because herbivores cannot use host movement for passive dispersal. Since there are a handful of studies investigating the genetic structure of micropredators, I reviewed past literature to test these hypotheses.

First, I collected 103 studies covering 166 animal species that provided the genetic structure information of free-living, micropredatory, herbivorous, and parasitic species (50, 55, 23 and 38, respectively) from as closely related taxa as possible. Due to bi-parental inheritance and higher marker resolution, I collected only microsatellite data. In total, I was able to collect 76 measurements of genetic distance (F_{st}) from species in multiple clades (17 separate orders) that could be compared (Table 4-1). Based on the methods of Mazé-Guilmo et al. (2016), I recorded a variety of different ecological categories for each species (see Chapter 2) to compare the genetic divergence among micropredators, herbivores, parasites, and free-living species. For each species I recorded a number of criteria that are likely to influence the population structuring of animals. Due to the low mitochondrial sample size, I decided to only analyze the microsatellite data. I plotted the linearized genetic distance (F_{st} /(1- F_{st})) against log-transformed geographic distance for each species based on whether it was a parasite, micropredator, herbivore, or free-living (Fig. 4-1). I also performed a generalized linear mixed model (GLMM) fit by restricted maximum likelihood (REML) in R 3.5.1 (R Core Team 2018), with the linearized F_{st} as the response variable, log geographic distance (km), tactic (parasite, micropredator, herbivore, or free-living), body size (mm), and dispersal mode (walking, jumping, swimming, or flight) as the explanatory variables. To remove the phylogenetic constraint I used clade (order) as a random effect.

Results showed that, while some trends are observable, such as parasites having the lowest F_{st} values, statistically, no clear effect of trophic strategy (i.e. micropredators, parasites, herbivore, and free-living animals) was detected (GLMM, $p = 0.13$, Fig. 4-1). I also found no significant relationship between any explanatory variable and F_{st}, though there were some slight positive correlations between linearized F_{st} and log distance (GLMM, $p = 0.13$) and the dispersal mode swimming (GLMM, $p = 0.15$).

The lack of any significant trend may be partly due to large variations within trophic strategies, as well as a relatively small sample size. For example, terrestrial and freshwater leeches tend to have very strong genetic divergence (Liu et al. 2016; Morishima et al. 2019; Qian and Davies 1996), which is also consistent with my study, but another species that attaches to the eyes of seabirds was genetically homogenous at large geographic scales (>1000km)(Nakano et al. 2020), because of long-distance dispersal via avian migration. In addition, it was also very difficult to find data from appropriate groups for comparison (i.e. clades containing all four different trophic strategies), or finding replicates in clades where micropredation had evolved multiple times.

The large variations of genetic divergence among and within micropredators probably result from the variations in the degree of passive dispersal. Passive dispersal of micropredators via prey movement should depend on contact time with their prey and is highly variable (Fig 4-2); from seconds in the case of many biting flies or cookiecutter sharks, to hours in the case of leeches. Some micropredators spend even days or weeks in contact with their prey, such as wintering ticks or migrating lamprey. Other factors such as body size and generation time can have both a positive and negative effect on dispersal, since it limits the distance and time possible for dispersal when compared with larger free-living species (Blasco-Costa and Poulin 2013), but also increase the effective population size and efficiency of passive dispersal (De Bie et al. 2012; Jenkins et al. 2007). Based on the results of my leech study, even in micropredators with relatively long attachment times to larger more mobile prey species, their ability to utilize passive dispersal is limited or non-existent. The greatest dispersers, I would argue, are species that can utilize the advantages of both active and passive dispersal, possessing both high vagility themselves and long host interaction, while still maintaining low prey specialization. The louse flies, for example, demonstrate this and in genetic studies conducted in the Galapagos Islands, louse flies frequently displayed less genetic structuring between islands than even their avian prey (Levin and Parker 2013; Whiteman et al. 2007). In general, the effects of passive dispersal on micropredator dispersal appear to be highly species dependent, adding further need for a robust data set with appropriate replicates and within clade comparability.

DISPERSAL AND POPULATION DYNAMICS OF MICROPREDATORS

Here, I would like to link the dispersal and ecological characteristics to the population dynamics of micropredators. As discussed in chapter 2, many micropredators play significant roles as vectors of diseases and pathogens and therefore population dynamics is especially relevant to their control.

As a result of frequent prey switching and low specialization, micropredators can potentially disperse even more widely than their most vagile prey species. Evidence for such dispersal is cited in a variety of studies (Dudaniec et al. 2008; Levin and Parker 2013; Mazé-Guilmo et al. 2016; McCoy et al. 2003; McCoy et al. 2016; Whiteman et al. 2007), finding unusually high dispersal in some micropredator or generalist parasite species. This pattern is often explained through such species using alternate prey/host reservoirs, resulting in higher dispersal and more frequent gene flow between populations than either specialist parasites or free-living predators (Levin and Parker 2013; McCoy et al. 2003; Whiteman et al. 2007).

More significant for micropredator dispersal and population stability than prey population dynamics, should be key environmental factors necessary for their survival (i.e. mosquitoes and still water), and this environmental impact should increase as the micropredator's interaction with its prey decreases (Fig. 4-2 and some evidence in Phillips 2012). That is, longer interaction time with prey individuals, decreases the importance of environmental factors on micropredatory species, though never to the same degree as parasites, and causes micropredator population structure to more closely resemble that of their most vagile prey (Mazé-Guilmo et al. 2016; McCoy et al. 2003;).

Fleas provide an excellent example, as they are broken into two groups based on the relative time adults are in direct contact with their prey. Species that spend a greater proportion of their time on their prey are referred to as "fur fleas", whereas species which spend most of their adult stage in the prey's nest environment are called "nest fleas" (van der Mescht et al. 2015). Nest fleas are more likely to be impacted by environmental conditions than fur fleas, additionally as they are tied to an environment type, they are also less capable at dispersing than fur fleas which can better utilize prey movement to travel (van der Mescht et al. 2015). However, due to their closer relationship with their prey, fur fleas also tend to have a narrower range of prey species than nest fleas, which tend to be more generalist.

Being able to feed multiple times and at considerable densities on the same prey individual, allows for large populations of micropredators to remain stable even if prey density and prey populations are relatively low or transient in nature (i.e. migratory animals). It can be predicted that unlike parasite and predator populations, which are directly tied to host/prey population size and density, micropredator populations will be mostly independent of prey population as a factor limiting their population size, and will instead be limited by key environmental conditions.

Control of many micropredator populations has proven difficult or impossible in the field, since directly interrupting their lifecycle by targeting easier to manage species, like in the case of parasite control, is not possible. Thus, micropredators need to be targeted for management in the same way as a free-living species, which often due to their size and numbers makes controlling their populations difficult. The most widely known example of this is the current range expansion of several species of Ixodid ticks capable of carrying Lyme disease, as they have been expanding northward with the changing global climate (Bouchard et al. 2013; Leighton et al. 2012; Sonenshine 2018).

EXPANDING ON MICROPREDATION AS A TROPHIC STRATEGY

A common issue with categorizing micropredators as separate from other trophic strategies is the large degree of variation in an otherwise relatively restricted number of taxa. As previously discussed, micropredators have been defined as animals that consume small or "micro" portions of their prey relative to body size (Lafferty and Kuris 2002; Lafferty et al. 2015). Other than the criteria outlined by Lafferty and Kuris (2002), there are other ways of separating consumer modes. For example, the number of consumer attacks while questing, relationship time, and relationship intimacy between enemies and victims are other metrics used to differentiate micropredators from predators and parasites (Lafferty et al 2015; Pollock et al 2021; Raffel et al 2008). As previously discussed, these points are particularly important when considering the dispersal among consumer populations, and if it reflects the dispersal of victims. To expand on the 4 dichotomies described by Lafferty and Kuris (2002), I would propose adding motile or sessile prey as an additional category to distinguish trophic strategies. Doing so would allow for the separation of micropredators and herbivores, as well as distinguish scavengers, detritivores, and decomposers from the existing trophic strategies.

Despite the advantages micropredation has in comparison to parasitism with regard to the relative lack of adaptations necessary to engage in it, obligate micropredators are taxonomically rare by comparison. If we include predators or scavengers that occasionally engage in micropredation and herbivores, micropredation is very common throughout the animal kingdom, but obligate micropredators of mobile organisms are only found among the Chordates, Molluscs, Arthropods, and Annelids, though there are undoubtedly more. As discussed, micropredation requires few adaptations from free-living ancestors, and we can expect that some parasite lineages also had micropredatory ancestors at one point (Kearn 2004). I would argue that micropredation of mobile organisms is a relatively high risk low return strategy. Micropredators hunt their prey like predators, but affect their prey like parasites (Lafferty et al. 2015), that is to say take only a proportionately small amount of the prey's resources per feeding. In scale-eating fishes for example, the small resource intake relative to the energy expenditure per attack is a reason for their reduced size compared to their predatory relatives (Janovetz 2005). Additionally, as prey are generally many times larger than the micropredator, each feeding comes at a considerable risk (Martin and Wainwright 2013). Another possible risk is that no viable prey is within the micropredators foraging range for a time, adding a degree of uncertainty in finding meals. It is argued that this is the primary reason for altruistic food sharing in vampire bats (Fenton 1992), which are known to share blood-meals with roost-mates that were unsuccessful in finding prey. By comparison, micropredators of sessile organisms like herbivores do not require complex hunting strategies, and once prey is discovered they have a stable source of nourishment. This is the major advantage of parasitism as well, which is why parasites co-evolve close relationships with only a few potential hosts in order to maximize resource efficiency over time. On the other hand free-living predators also have to find and hunt prey each time they feed, but unlike micropredators they acquire far more of their prey's resources from a successful hunt. To reduce the risk of injury and energy costs during attacks, predators (solitary predators especially) are often of similar in size or larger than their preferred prey.

Despite being phylogenetically restricted, micropredators are among the most successful species in terms of range and biomass, and can have a significant impact on prey populations. Many of the most famous micropredators are carriers of harmful parasites that shape the distribution and evolution of prey species. One only has to think of the bubonic plague spread by fleas during the Middle Ages in Europe, for an example of the effect micropredators can have on prey populations. Herbivores are no different in this regard, and the diversity of agricultural pests whether through the diseases they carry or the swarms they can create, have been recorded since antiquity.

CONCLUSION

Overall, I concluded that micropredation is a relatively common trophic strategy, though not as common as predation and parasitism, in many ecosystems. Micropredation appears to lack the extensive evolutionary adaptations required for a parasitic lifestyle, and seems to have evolved from various free-living feeding behaviours, such as blood, fruit, or nectar feeding, independently on multiple occasions. Micropredators possess unique ecological characteristics, sometimes shared with either parasites or predators, situating them as a middle ground between the two. And while they superficially seem to bear a close resemblance to parasites, their ecological characteristics have more in common with free-living predators. We should incorporate and distinguish them as a legitimate ecological strategy, including separate models and methods of studying them, so as not to have them mistakenly grouped together with parasites. Micropredators constitute some of the most influential species ecologically, medically, and economically as a result of their role as vectors for diseases in humans and other animals. Ticks and mosquitoes are a growing concern in many regions around the world, as range expansions related to changes in climate are allowing them to spread diseases to prey populations that lack resistances.

Additionally, I hope my case study of the leech-fish system inspires studies on other micropredator-prey relationships.

Tables

Table 4-1 Literature review reference data for all species that provided microsatellite F_{st} values. Categories and methodology based on Mazé-Guilmo et al. (2016). For the category Host/Prey Dispersal Mode, "sessile" was included to differentiate between herbivores and other trophic strategies. Host/Prey Dispersal Spectrum refers to feeding specialization or generality.

Species	**Clade**	**Tactic**	**Sexual Mode**	**% Free-living Stages**	**Body Size (mm)**	**Dispersal Mode**	**Host/Prey Dispersal Mode**	**Host/Prey Dispersal Spectrum**	**Region**	**Mean Site Distance (km)**	**log Distance (km)**	**Mean *Fst***	***Fst*/(1-*Fst*)**
Buteo galapagoensis	Accipitriformes (Order)	free-living	strictly gonochoric sexual	100	500	flight	walking	strongly generalist	Galapagos	130	2.11	0.35	0.54
Orchomenella franklini	Amphipoda (Order)	free-living	strictly gonochoric sexual	100	7	walking	sessile	strongly generalist	Antarctica	1000	3.00	0.09	0.09
Erpobdella punctata	Arhynchobdellida (Order)	free-living	hemaphrodite sexual	100	230	swimming	swimming	strongly generalist	U.S.A. Arizona	150	2.18	0.74	2.85
Procyon lotor	Carnivora (Order)	free-living	strictly gonochoric sexual	100	550	walking	walking	strongly generalist	U.S.A. Indiana	18	1.26	0.03	0.03
Myotis myotis	Chiroptera (Order)	free-living	strictly gonochoric sexual	100	85	flight	flight	strongly generalist	Switzerland and Italy	320	2.51	0.29	0.40
Myotis bechsteinii	Chiroptera (Order)	free-living	strictly gonochoric sexual	100	48	flight	flight	strongly generalist	Switzerland and Italy	320	2.51	0.39	0.63
Variabilichromis moorii	Cichliformes (Order)	free-living	strictly gonochoric sexual	100	100	swimming	swimming	strongly generalist	Lake Tanganyika, Zambia 18 km (estimate)	26	1.41	0.10	0.11
Liponeura cinerascens	Diptera (Order)	free-living	strictly gonochoric sexual	100	8	flight	sessile	strongly generalist	West, Central Europe	1000	3.00	0.27	0.36
Orius strigicollis	Hemiptera (Order)	free-living	strictly gonochoric sexual	100	2	flight	walking	strongly generalist	Japan	500	2.70	0.12	0.14
Appasus japonicus	Hemiptera (Order)	free-living	strictly gonochoric sexual	100	19	flight	swimming	strongly generalist	Japan and Korea	600	2.78	0.52	1.06
Abedus herberti	Hemiptera (Order)	free-living	strictly gonochoric	100	25	swimming	swimming	strongly generalist	U.S.A	100	2.00	0.13	0.15

			sexual										
Macrolophus pygmaeus	Hemiptera (Order)	free-living	strictly gonochoric sexual	100	4	flight	walking	strongly generalist	South Europe	3000	3.48	0.13	0.14
Macrolophus pygmaeus	Hemiptera (Order)	free-living	strictly gonochoric sexual	100	4	flight	walking	strongly generalist	Central and South Europe	3000	3.48	0.25	0.33
Halobates japonicus	Hemiptera (Order)	free-living	strictly gonochoric sexual	100	6	walking	swimming	strongly generalist	Japan	1	0.00	0.14	0.16
Jaera albifrons	Isopoda (Order)	free-living	strictly gonochoric sexual	100	3	walking	sessile	strongly generalist	France	200	2.30	0.14	0.16
Jaera praehirsuta	Isopoda (Order)	free-living	strictly gonochoric sexual	100	3	walking	sessile	strongly generalist	France	200	2.30	0.15	0.18
Septemserolis septemcarinata	Isopoda (Order)	free-living	strictly gonochoric sexual	100	25	walking	sessile	strongly generalist	South Atlantic/Antarctic	2000	3.30	0.12	0.14
Isocladus armatus	Isopoda (Order)	free-living	strictly gonochoric sexual	100	15	swimming	sessile	strongly generalist	New Zealand	1000	3.00	0.40	0.67
Serolis paradoxa	Isopoda (Order)	free-living	strictly gonochoric sexual	100	25	walking	sessile	strongly generalist	Patagonia and Falkland Islands	600	2.78	0.24	0.32
Jaera albifrons	Isopoda (Order)	free-living	strictly gonochoric sexual	100	3	walking	sessile	strongly generalist	France	400	2.60	0.01	0.01
Isocladus armatus	Isopoda (Order)	free-living	strictly gonochoric sexual	100	20	swimming	walking	strongly generalist	New Zealand	700	2.85	0.18	0.22
Neoseiulus womersleyi	Mesostigmata (Order)	free-living	strictly gonochoric sexual	100	2	walking	walking	strongly generalist	Japan, Mie	0.1	-1.00	0.03	0.03
Lampetra planeri	Petromyzontiformes (Order)	free-living	strictly gonochoric sexual	100	130	swimming	swimming	strongly generalist	France	390	2.59	0.13	0.15
Cynomys ludovicianus	Rodentia (order)	free-living	strictly gonochoric sexual	100	400	walking	sessile	strongly generalist	U.S.A. Montana	46	1.66	0.20	0.25
Thomomys bottae	Rodentia (order)	free-living	strictly gonochoric sexual	100	230	walking	sessile	strongly generalist	U.S.A. New Mexico	200	2.30	0.24	0.31
Haemadipsa	Arhynchobdelli	micropr	hemaphrodit	100	30	walking	walking	strongly	Japan	600	2.78	0.34	0.51

japonica	da (Order)	edator	e sexual					generalist					
Whitmania pigra	Arhynchobdellida (Order)	micropredator	hemaphrodite sexual	100	30	walking	walking	strongly generalist	China	1500	3.18	0.54	1.16
Hirudo nipponica	Arhynchobdellida (Order)	micropredator	hemaphrodite sexual	100	30	walking	walking	strongly generalist	China	1500	3.18	0.21	0.27
Poecilobdella manillensis	Arhynchobdellida (Order)	micropredator	hemaphrodite sexual	100	30	walking	walking	strongly generalist	China	1500	3.18	0.15	0.18
Anopheles gambiae s.s.	Diptera (Order)	micropredator	strictly gonochoric sexual	100	4.5	flight	any	strongly generalist	Northwestern Africa	580	2.76	0.08	0.09
Anopheles gambiae	Diptera (Order)	micropredator	strictly gonochoric sexual	100	4.5	flight	any	strongly generalist	Africa	4350	3.64	0.10	0.11
Glossina morsitans submorsitans	Diptera (Order)	micropredator	strictly gonochoric sexual	100	10	flight	walking	strongly generalist	Northwest, East, Southeast Africa	4697	3.67	0.17	0.20
Glossina morsitans centralis	Diptera (Order)	micropredator	strictly gonochoric sexual	100	10	flight	walking	strongly generalist	Northwest, East, Southeast Africa	4697	3.67	0.18	0.22
Glossina morsitans morsitans	Diptera (Order)	micropredator	strictly gonochoric sexual	100	10	flight	walking	strongly generalist	Northwest, East, Southeast Africa	4697	3.67	0.19	0.23
Glossina pallidipes	Diptera (Order)	micropredator	strictly gonochoric sexual	100	10	flight	walking	strongly generalist	Northwest, East, Southeast Africa	4697	3.67	0.31	0.45
Philornis downsi	Diptera (Order)	micropredator	strictly gonochoric sexual	66	6	flight	flight	strongly generalist	Galapagos	85	1.93	0.02	0.02
Bactrocera dorsalis	Diptera (Order)	micropredator	strictly gonochoric sexual	100	8	flight	sessile	strongly generalist	South Asia	1000	3.00	0.26	0.36
Hishimonus phycitis	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	5	jumping	sessile	strongly generalist	United Arab Emirates and Southern Iran	600	2.78	0.06	0.06
Stenotus rubrovittatus	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	5	flight	sessile	weakly generalist	Japan	150	2.18	0.02	0.02
Orthops palus	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	7	flight	sessile	strongly generalist	Reunion Island	30	1.48	0.03	0.03
Scaphoideus titanus	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	5	jumping	sessile	weakly generalist	North East U.S.A	50	1.70	0.04	0.04
Scaphoideus	Hemiptera	micropr	strictly	100	5	jumping	sessile	weakly	Western Europe	400	2.60	0.05	0.05

titanus	(Order)	edator	gonochoric sexual					generalist					
Pseudococcus viburni	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	3	walking	sessile	strongly generalist	France, Italy, Chile, and New Zealand	11750	4.07	0.27	0.36
Creontiades dilutus	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	8	flight	sessile	strongly generalist	Australia	2100	3.32	0.12	0.14
Lygus lineolaris	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	6	flight	sessile	strongly generalist	U.S.A	10	1.00	0.05	0.05
Triatoma infestans	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	35	flight	walking	strongly generalist	Peru	20	1.30	0.08	0.09
Cimex lectularius	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	6	walking	walking	strongly generalist	U.S.A. East Coast	1000	3.00	0.68	2.13
Cimex hemipterus	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	6	walking	walking	strongly generalist	Malaysia and Singapore	350	2.54	0.28	0.39
Rhodnius pallescens	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	30	flight	walking	strongly generalist	Ecuador	100	2.00	0.01	0.01
Cimex lectularius	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	6	walking	walking	strongly generalist	France	600	2.78	0.56	1.25
Cimex lectularius	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	6	walking	walking	strongly generalist	U.S.A	150	2.18	0.49	0.95
Cimex lectularius	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	6	walking	walking	strongly generalist	U.S.A	650	2.81	0.36	0.56
Cimex hemipterus	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	6	walking	walking	strongly generalist	Malaysia	300	2.48	0.12	0.14
Triatoma dimidiata	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	20	flight	walking	strongly generalist	Guatemala	5	0.70	0.03	0.03
Triatoma infestans	Hemiptera (Order)	micropredator	strictly gonochoric sexual	100	35	flight	walking	strongly generalist	Argentina	800	2.90	0.14	0.16
Ixodes texanus	Ixodida (Order)	micropredator	strictly gonochoric sexual	0	3.5	walking	walking	strongly generalist	U.S.A. Indiana	18	1.26	0.00	0.00

Ixodes uriae	Ixodida (Order)	micropredator	strictly gonochoric sexual	100	3.5	walking	walking/flight	strongly generalist	North Atlantic Ocean	2400	3.38	0.03	0.04
Ixodes uriae	Ixodida (Order)	micropredator	strictly gonochoric sexual	100	3.5	walking	walking/flight	strongly generalist	North Atlantic Ocean	2400	3.38	0.06	0.06
Ctenolabrus rupestris	Labriformes (Order)	micropredator	strictly gonochoric sexual	100	180	swimming	swimming	strongly generalist	Eastern North Atlantic	2000	3.30	0.04	0.04
Symphodus melops	Labriformes (Order)	micropredator	strictly gonochoric sexual	100	150	swimming	swimming	strongly generalist	Eastern North Atlantic	2000	3.30	0.09	0.09
Petromyzon marinus	Petromyzontiformes (Order)	micropredator	strictly gonochoric sexual	100	700	swimming	swimming	strongly generalist	Western North American	450	2.65	0.02	0.02
Lampetra fluviatilis	Petromyzontiformes (Order)	micropredator	strictly gonochoric sexual	100	350	swimming	swimming	strongly generalist	France	390	2.59	0.02	0.02
Petromyzon marinus	Petromyzontiformes (Order)	micropredator	strictly gonochoric sexual	100	700	swimming	swimming	strongly generalist	Lake Superior North America	100	2.00	0.04	0.04
Lampetra fluviatilis	Petromyzontiformes (Order)	micropredator	strictly gonochoric sexual	100	350	swimming	swimming	strongly generalist	West, Northwest, North Europe	2000	3.30	0.50	0.99
Oropsylla hirsuta	Siphonaptera (Order)	micropredator	strictly gonochoric sexual	33	2	jumping	walking	weakly generalist	U.S.A. Montana	46	1.66	0.06	0.06
Listropsylla agrippinae	Siphonaptera (Order)	micropredator	strictly gonochoric sexual	100	2	jumping	walking	strongly generalist	South Africa	630	2.80	0.37	0.58
Chiastopsylla rossi	Siphonaptera (Order)	micropredator	strictly gonochoric sexual	100	2	jumping	walking	strongly generalist	South Africa	630	2.80	0.51	1.05
Basilia nana	Diptera (Order)	parasite	strictly gonochoric sexual	50	6.5	host contact	flight	weakly generalist	Germany	10	1.00	0.01	0.01
Gasterophilus pecorum	Diptera (Order)	parasite	strictly gonochoric sexual	33	18	flight	walking	strongly generalist	China Xingjiang	180	2.26	0.04	0.04
Spinturnix myoti	Mesostigmata (Order)	parasite	strictly gonochoric sexual	0	2	host contact	flight	weakly generalist	Switzerland and Italy	320	2.51	0.01	0.01
Spinturnix bechsteini	Mesostigmata (Order)	parasite	strictly gonochoric sexual	0	2	host contact	flight	specific	Switzerland and Italy	320	2.51	0.23	0.30

Spinturnix bechsteini	Mesostigmata (Order)	parasite	strictly gonochoric sexual	0	2	host contact	flight	specific	Germany	10	1.00	0.23	0.30
Physconelloides spp.	Phthiraptera (Order)	parasite	strictly gonochoric sexual	0	3	host contact	flight	specific	Southern U.S.A. to Southern South America	1000	3.00	0.06	0.06
Columbicola spp.	Phthiraptera (Order)	parasite	strictly gonochoric sexual	0	3	host contact	flight	specific	Southern U.S.A. to Southern South America	1000	3.00	0.06	0.06
Geomydoecus actuosi	Phthiraptera (Order)	parasite	strictly gonochoric sexual	0	2.5	host contact	walking	specific	U.S.A. New Mexico	200	2.30	0.24	0.32
Degeeriella regalis	Phthiraptera (Order)	parasite	strictly gonochoric sexual	0	3	host contact	flight	specific	Galapagos	130	2.11	0.43	0.75

Figures

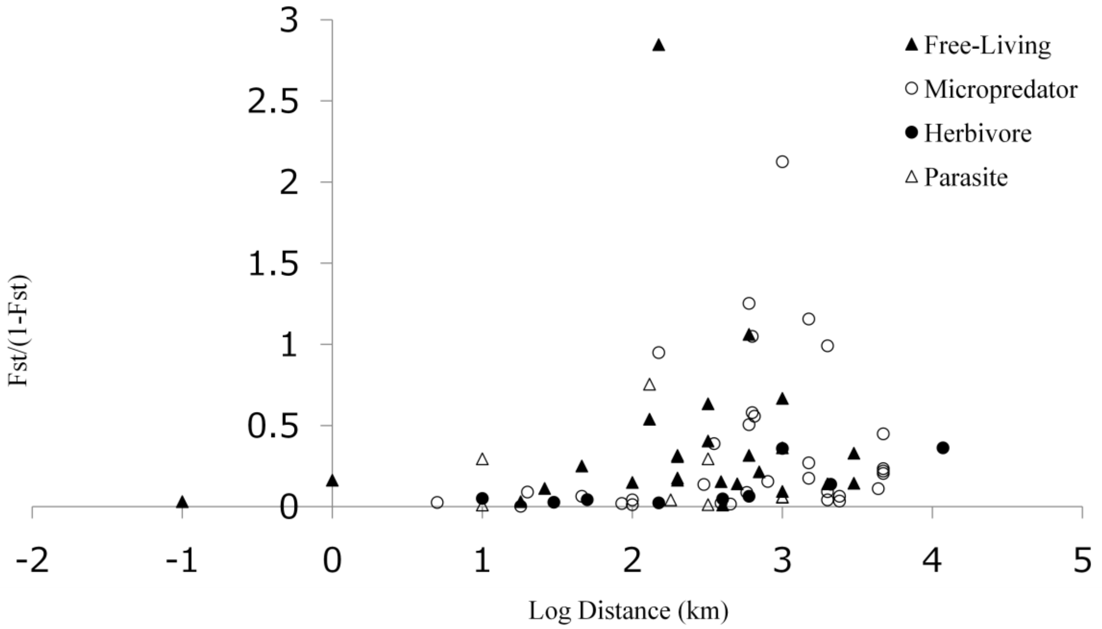

Fig. 4-1 Microsatellite F_{st} values of table 4-1 plotted for free-living, herbivore, micropredator, and parasite species by log distance. No significant correlation was found between F_{st} and log distance ($p = 0.13$) or any other category used in the GLMM, such as micropredator ($p = 0.48$) or herbivore ($p = 0.13$).

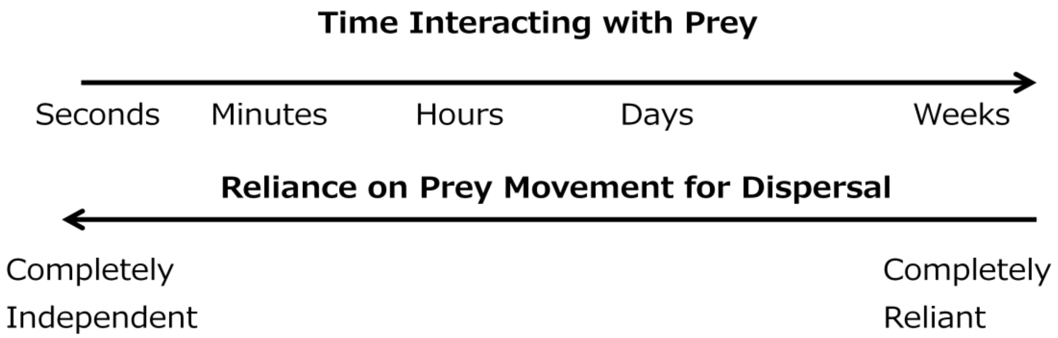

Fig. 4-2 Micropredator dispersal in relation to prey movement, as interaction time with prey increases so too should reliance on prey for micropredator dispersal.

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