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**Comparisons of host specificity between feather louse genera (Insecta: Phthiraptera: Philopteridae) parasitizing gulls (Aves: Laridae: *Larus*)**

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## ABSTRACT

Data from gene sequences and morphological structures were collected for the gull feather lice, *Saemundssonina lari*, *Quadraceps punctatus*, and *Q. ornatus*, parasitizing *Larus crassirostris* and *L. schistisagus*. *S. lari* was collected from both gull species, and no detectable morphological and genetic differences were found between the lice collected from the two different hosts. In contrast, *Q. punctatus* was only collected from *L. crassirostris* whereas *Q. ornatus* was only collected from *L. schistisagus*. The two *Quadraceps* species were genetically highly divergent, and body-size differences corresponding to the gull's body size (Harrison's rule) were also detected between them. Both *Quadraceps* species were collected from the interbarb of the remex or rectrix, and a match in body size between the louse and the interbarb space may be important for escaping from host preening defenses. In contrast, *Saemundssonina* is a head louse, inhabiting the finer feathers of the head and neck, which the bird cannot preen. A close match to host body size may be less important for lice in the head microhabitat. The differences in the pattern of host-specificity between *Saemundssonina* and *Quadraceps* on the two focal host species of this study were probably due to their different microhabitat preferences. More broadly, comparisons of the gene sequences of *S. lari* and *Q. punctatus* to those from other gull hosts showed that genetically almost undifferentiated populations of both species were distributed on wide range of gull species. Frequent interspecific hybridization of gulls is one possible factor that may allow these lice to maintain gene flow across multiple host species.

Key Words: *Saemundssonina*, *Quadraceps*, Harrison's rule, host switch, parasitic lice

## INTRODUCTION

Parasitic lice (Psocodea: Phthiraptera) are obligate parasites of mammals and birds and spend their entire lifecycle on the hosts. Their external structures are highly specialized to the ectoparasitic lifestyle (e.g., flattened body, short legs, absence of wings) and their mobility is extremely restricted (Johnson and Clayton, 2003). Usually, lice transmit to a new host individual only via direct contact between the hosts, such as during copulation (Hillgarth, 1996) or parental care (Clayton and Tompkins, 1994). Because of these constraints, parasitic lice frequently show strong host specificity, and many species of lice are restricted to single genus or species of host (e.g., Marshall, 1981). This strong host specificity makes the lice an ideal model system for studying the host-parasite coevolution and co-speciation (Johnson and Clayton, 2003).

This high host specificity notwithstanding, some lice show low host specificity and parasitize a wide range of host species (Price et al., 2003). Such a wide range of host species is generally thought to be achieved by some factors other than the direct contact between the hosts. For example, it is well known that the hippoboscids frequently transmit lice between different host bird species (phoresis: Keirans, 1975). In such cases, genetic structure of a louse species is known to reflect biogeography rather than the host distribution, probably restricted by the distributional range of hippoboscids (Johnson et al., 2002; Weckstein, 2004). However, even if lice successfully transmit to a different host species via phoresis or other means, a morphological mismatch between the host and louse may prohibit colonization of the lice on the new host species. For example, the wing lice of birds escape from host preening defenses by inserting themselves between the barbs of the wing feathers. One study found that the body width of the wing lice from pigeons and doves is strongly correlated with host body size, which dictates the width of the feather interbarb space (Johnson et al., 2005). A mismatch between louse body width and the host's interbarb space prohibits the louse escaping from host preening and makes it difficult to colonize host species that differ in body size from the native host.

Even in species of lice with a wide host range, molecular analyses sometimes reveal significant genetic divergence between lice on different host species. For example, species of both body and wing lice that parasitize different species of doves show considerable population structure between different host species (Johnson et al., 2002). A similar pattern has also been identified in the louse genus *Lunaceps* that parasitizes shore birds (Gustafsson

and Olsson, 2012). Deep genetic divergence within a louse species on a single host species, but in different geographic locations, has also been identified (Mizukoshi et al., 2012). These examples clearly show that it is sometimes inappropriate to evaluate the host-range of a louse species based on morphological information alone, which has been utilized for species-level taxonomy of lice.

*Saemundssonina lari* is one louse species with a very wide host range, recorded from 36 species across six genera of Laridae (gulls and their relatives) (Price et al. 2003). This species is of the head louse ecomorph (Johnson et al. 2012), possessing a triangular head and rounded body (Fig. 1A), and living in the head and neck regions of the bird using its mandibles to grip feather barbs. Gulls are also parasitized by a closely related genus, *Quadraceps* (Cruickshank et al. 2001; Smith et al. 2004; Price et al. 2003). This genus is as a whole recognized as a generalist (Johnson et al. 2012). However, species of the genus parasitizing gulls have an elongated head and body (Fig. 1BC), and they insert into the interbarb spaces of the flight feathers on the wing in a similar way to wing lice that use this behavior to escape preening (Clayton et al., 2003). Therefore, the species treated here exhibit the same escape behavior as the wing louse ecomorph found on other groups of birds. In general, species of *Quadraceps* show a much higher degree of host specificity than those of *Saemundssonina*. Four species, subdivided into many subspecies, of *Quadraceps* have been recorded from the gull genus *Larus* (Price et al., 2003).

In this study, we compare the morphological and genetic structures of *Saemundssonina* and *Quadraceps* collected from two species of gulls breeding in Japan: Black-tailed Gull (*Larus crassirostris*) and Slaty-backed Gull (*Larus schistisagus*). The former is smaller (ca. 47cm) than the latter (ca. 64cm) in body length, and both gulls are parasitized by *Saemundssonina lari*. However for *Quadraceps*, the Black-tailed Gull is host only to *Quadraceps punctatus* (also known to parasitize 26 other *Larus* species) whereas the Slaty-backed Gull is host only to *Q. ornatus* (also known to parasitize six other *Larus* species). In this study we address (1) are there any hidden morphological or genetic differences in *S. lari* parasitizing different host species and, if not, (2) what causes the difference in host specificity between the two closely related louse genera using different microhabitats of the same two host species.

## **MATERIALS AND METHODS**

All samples were collected at Teuri Island, an island located west to northern Hokkaido. This island is isolated around 28km from the mainland Hokkaido, and its circumference is about 3km. Both Black-tailed and Slaty-backed Gulls breed on this small island. Both head (*Saemundssonina*) and wing lice (*Quadriceps*) were collected from living and dead birds under the permits from the Agency for Cultural Affairs and from Ministry of the Environment. Louse specimens were searched for by eye and picked up using a forceps. Lice were killed and preserved in 99.5% ethanol. For *Saemundssonina lari*, 16 males and 16 females collected from five different Black-tailed Gulls, and 10 males and 14 females collected from 11 Slaty-backed Gulls. For *Quadriceps*, 8 males and 7 females of *Q. punctatus* were collected from four different Black-tailed Gulls, and 7 males and 9 females were collected from six Slaty-backed Gulls.

Partial sequences (334 bp) of the mitochondrial cytochrome oxidase subunit I gene (COI) were amplified and sequences using primer pairs L6625 + H7005 (Hafner et al., 1994). Protocols for total DNA extraction, PCR, sequencing and alignment followed Yoshizawa (2004). However, initial PCRs only provided faint amplification for *Q. ornatus*. Therefore, the PCR product was cloned prior to sequencing using pGemT Easy Vector system (Promega, Madison, Wisconsin) following manufacture protocols. GenBank Accession Numbers for the presently obtained sequences are AB909115–AB909120. Voucher specimens are stored in Hokkaido University Insect Collection. Sequences for the outgroups (*Rallicola*) and some *Saemundssonina* spp. and *Quadriceps* spp., including those of *S. lari* and *Q. punctatus* from other host gulls, were also obtained from GenBank.

Phylogenetic analyses were conducted under maximum parsimony and maximum likelihood criteria using PAUP\* 4.0b10 (Swofford, 2002). For MP analysis, all characters were equally weighted, and heuristic searches were conducted with 100 random starting trees and TBR branch swapping. For ML analysis, a single heuristic search with TBR branch swapping was performed using a NJ tree as a starting tree. The best fit substitution model for ML analysis was estimated by hierarchical Likelihood Ratio Test as implemented in jModelTest 2.1.1 (Darriba et al., 2012), and the HKY+G model was selected. Detailed parameters of the substitution model are described in the data matrix available as an Online Supplementary Data. Bootstrap support values were calculated from 100 pseudo-replicates under both MP and ML using PAUP\*. Parameter settings for bootstrap analyses were identical to those adopted for MP and ML tree estimation.

The voucher specimens from the DNA extraction were used for morphological examination. The cleared exoskeleton was mounted on a HS slide (Shirayama et al., 1993) with Euparal. Examination was conducted with an Olympus BX40 light microscope, and the lengths of precornal head width, temporal head width, metathoracic width, abdominal width (5th segment), head length, total body length minus head length, groove width, and paramere length (a part of male genitalia: Fig. 1A) were measured (Fig. 1). Because male genital structures of two *Quadraceps* species differed morphologically (Fig. 1BC), and the purpose of the paramere measurements for *Saemundssonina* was to determine potentially hidden morphological differences between host races, paramere length was only measured for *Saemundssonina*. Statistical analysis (*t*-test) was conducted using JMP 9.1 (SAS, 2011).

## RESULTS

### Molecular analyses

From *Saemundssonina lari* collected in Teuri Island, three haplotypes were detected, and the maximum uncorrected *p*-difference between them is only 0.6% (Fig. 2). All haplotypes clustered into a single clade, and, except for haplotype 2 unique to the Slaty-backed Gull (*n*=4), all other haplotypes were present in lice from both gull species. Four sequences of *S. lari* collected from other gull species and downloaded from GenBank also clustered closely with the *S. lari* sequences obtained from Teuri Island. In particular, the sequences obtained from *S. lari* parasitizing Herring Gull (*L. argentatus*: Europe) and Kelp Gull (*L. dominicanus*: South Hemisphere) showed completely identical sequences with the haplotype 1 of *S. lari* from Teuri Island (Fig. 2). Even *S. lari* obtained from geographically distant gull species, Gray-headed Gulls (*L. cirrocephalus*: Africa and South America) and Lesser Black-backed Gull (*L. fuscus*: Europe), showed at a maximum only 0.9% sequence divergence from those of Teuri Island.

The two species of *Quadraceps* were host specific, at least within Teuri Island (Fig. 2). They are genetically highly divergent at about 22% uncorrected sequence divergence. Each species composed a clade with either a single haplotype (*Q. punctatus*) or two haplotypes with extremely low genetic divergence (0.3%: *Q. ornatus*). Intraspecific sequence difference within *Q. punctatus* was low (0-1.00%), even when including the sequences from the species on other gull hosts (i.e. *Q. punctatus* from *L. cirrocephalus* from Africa and South America, *L. dominicanus* from the South Hemisphere, and *L. californicus* from western North

America).

### **Morphological analyses**

Significant differences in body size between males and females were detected for all species, and, in all cases, females were about 20% larger than males (Tables 2-3).

*Saemundssonina* collected from different species showed no significant difference in most measurements, including groove width (a functionally relevant character used in the process of attaching to feather barbs) and paramere length (a male genitalic character) (Table 1). However, significant differences were detected in abdominal width and total body length minus head length of females (Table 1). The two *Quadriceps* species from the two gull species were significantly different in many features, including genital structure (Fig. 1BC), such that their independent species status can be confirmed based on morphology alone. Significant differences existed between the two *Quadriceps* species in the precornal head width, temporal head width, metathoracic width, and total body length minus head length, whereas no significant differences were detected in head length, and abdominal width (Table 2). Measurements showing significant differences were always larger in *Q. ornatus*, which occurs on the larger host (*L. schistisagus*), except that total body length minus head length showed opposite trend (Table 2).

### **DISCUSSION**

Genetic analysis indicates that *Saemundssonina lari* collected from two host gulls breeding on Teuri Island represent a single species with low genetic divergence. Although significant differences are identified in the abdominal width and length of female *S. lari* collected from two host gulls, the abdomen has wide membranous regions (Fig. 1A) and thus its shape can be altered easily by some extrinsic factors, such as egg maturation or artifacts during slide preparations. As far as the heavily sclerotized structures are concerned, no significant morphological difference is identified between the lice from different host species. Therefore, morphological evidence also suggests that populations of these lice on the two different host species are conspecific. In contrast, the *Quadriceps* on the two gulls breeding on Teuri Island are clearly different species based on both morphological and molecular evidence. These results indicate that two closely related louse genera parasitizing two gull species breeding on the same island differ dramatically in their patterns in host specificity.

All *Quadraceps* examined here were collected from the interbarb spaces of the remex or rectrix (i.e. flight feathers), whereas *S. lari* were collected from the downy part of the head and neck regions. Therefore, although Johnson et al. (2012) classified the genus *Quadraceps* as generalist (lice with no specific microhabitat preference on the host's body), at least the two *Quadraceps* species examined here can be regarded as wing lice. That is, they exhibit the same escape behavior that other wing lice use to avoid preening (Clayton et al., 2003). *Saemundssonina* can be confidently classified as the head louse, as also suggested by Johnson et al. (2012). These microhabitat differences are likely to be the largest factor influencing the difference in patterns of host specificity of the two louse genera parasitizing these two gull species on Teuri Island. Wing lice escape from host's preening by inserting their body between the interbarb space of the flight feathers. Therefore, any mismatch between the width of the louse and the feather interbarb space increases the risk of being preened off the host (Clayton et al., 2003; Bush and Clayton, 2006). In the case of the *Quadraceps* species examined here, lice on different host gulls show significantly different body widths, and the louse on the larger host exhibited a larger body size (Table 2), showing good correspondence between host and parasite body size. Although the abdominal length shows the opposite trend (larger in the louse on the smaller host), different from the body width, body length is less likely to cause a mismatch between louse body size and feather interbarb space, because lice insert with their central body axis parallel to the feather barbs. A correlation between host and parasite body size, generally known as Harrison's rule (Harrison, 1915; Johnson et al., 2005), probably prevents parasites from shifting to a new host with a greatly different body size. In contrast, Harrison's rule has not been detected in body lice, which burrow in feather down (Johnson et al., 2005). This also agrees with the present observation, because *S. lari* inhabit the downy parts of the bird's head and neck feathers, and no morphological difference was detected between *S. lari* collected from two gull species. However, it should also be noted that Timmermann (1951) recognized head size variation of *S. lari* roughly following Harrison's rule when host size is smaller than *L. canus* (wing length ca. 36cm), although the comparisons were not subjected to statistical tests.

The other question is what mechanisms can homogenize populations of *S. lari* parasitizing different gull species. Generally, lice transmit to a new host individual only via direct contact of host animals, such as breeding and mating, whereas interspecific direct contact between different host species is limited. Phoresis mediated by the hippoboscid flies



is a well known factor which frequently causes host-switching of bird lice. However, no species of hippoboscid fly has been recorded from the Japanese gulls (Maa, 1969; Keirans, 1975) and thus the phoresis is an unlikely mechanism. Teuri Island is utilized as a breeding site for many seabirds (including Rhinoceros Auklet, Japanese Cormorant and so on), and their density on the island is also very high. Slaty-backed Gulls sometimes prey on chicks of Black-tailed Gulls (Watanuki, 1988). This probably provides frequent direct contact between different host species and a possible dispersal opportunity for lice. This may include not only dispersal from smaller to larger gulls, but also larger to smaller because the adults of smaller gulls will attack predatory gulls. Host-size differences probably do not prevent establishment of head louse genus *Saemundssonina* (Johnson et al., 2005), but a mismatch between the louse body size and the feather interbarb probably prevent establishment of the wing louse genus *Quadriceps* on a novel host (Bush and Clayton, 2006). This local factor may also extend different breeding sites.

Several other factors may also enable lice to disperse between different species of gulls when host individuals contact each other during the breeding season. These include encounters during fishing frenzies, territorial fights, and theft of nesting material. Hybridization between gull species may also lead to dispersal of lice between species, and such hybrids are known between many different species of gulls. For example, the white-headed gull species-group consists of 18 species, including Slaty-backed Gull, and different species hybridize quite frequently (Liebers et al., 2004; Olsen and Larsson, 2004; Pons et al., 2005). Although the distributional range of Slaty-backed Gull is restricted to Japan and neighboring regions, some gulls in the species-group show very wide distributional ranges (Olsen and Larsson, 2004). For example, hybridizing pairs of Slaty-backed Gull and Glaucous-winged Gull (*L. glaucescens*) have been observed in Rishiri and Rebun Islands, located only ca. 75km north of Teuri Is. (Kosugi, 2003; Kazama et al., 2011). The distribution of the Glaucous-winged Gull includes both Asia and North America, where it overlaps with a different suite of gull species. It is likely that such widely distributed gulls spread their lice widely and facilitate mixing of louse populations even between geographically distant host species.

Although these dispersal opportunities occur locally, worldwide, *S. lari* only shows at most 0.9% sequence difference, even between individuals collected from phylogenetically distant host species (Pons et al., 2005) whose geographic ranges are also very separated (e.g.,

Japan and Africa, South America). *Q. punctatus* also shows low genetic divergences within the species, even though louse samples were from widely separated host gulls. Widespread gull species in combination with gull migration may facilitate a more global homogenization of louse populations. During winter, many species of gulls roost together in large flocks. These communal winter roosts include species of gulls that do not normally overlap during the breeding season. Ongoing gene flow of *Saemundssonina* among many widely distributed hosts is probably possible. However, a mismatch between parasite and host body sized likely prevents *Quadraceps* from establishing across such a wide range of host species. Interestingly, the host species of *Q. punctatus* examined here are mostly smaller gulls but also include a larger one (*L. dominicanus*: about the same size as Slaty-backed Gull). Transmission of small lice to larger host may not prevent louse survival over shorter timescales (Bush and Clayton, 2006). It might also be possible that the population of *Q. punctatus* on *L. argentatus* is transient but, according to Timmermann (1971), *Q. punctatus* is quite frequently found on *L. argentatus* as well as *Q. ornatus*. Because a single host individual rarely host two species of lice, Timmermann (1971) argued that different populations of a host species host different species of *Quadraceps*, and distributional ranges of *Quadraceps* species are rather restricted by climates than host species. Further morphological and genetic analyses of *Quadraceps* collected from much wider range of gull species are needed to uncover the factors which allow/prevent dispersal and establishment of gull wing lice.

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**Table 1.** Measurements of *Saemundssonina lari* collected from two gull species

	from <i>L. crassirostris</i> <i>n</i> =16 each (male, female)	from <i>L. schistisagus</i> <i>n</i> =10 (male), 14 (female)	P ( <i>t</i> -test)
PCHW-male	0.3581±0.0091	0.3580±0.0244	0.9852
PCHW-female	0.3963±0.0152	0.3900±0.0180	0.9187
THW -male	0.5831±0.0135	0.5830±0.0157	0.9830
THW-female	0.5606±0.0112	0.6479±0.0239	0.6818
MW-male	0.4619±0.0138	0.4450±0.0344	0.0902
MW-female	0.5125±0.0148	0.5000±0.0332	0.1851
AW-male	0.8150±0.0239	0.7830±0.0881	0.1782
AW-female	0.9667±0.0305	0.9150±0.0720	0.0171*
HL-male	0.5506±0.0152	0.5450±0.0165	0.3840
HL-female	0.5875±0.0161	0.5867±0.0164	0.8934
TL-HL-male	1.2111±0.0385	1.1790±0.0814	0.1921
TL-HL-female	1.4813±0.0427	1.3817±0.1284	0.0073**
GW-male	0.1272±0.0066	0.1240±0.0057	0.2182
GW-female	0.1331±0.0070	0.1329±0.0099	0.9321
PL-male	0.2900±0.0035	0.2910±0.0044	0.8606

Abbreviations: PCHW=precornal head width; THW=temporal head width; MW=metathoracic width; AW=abdominal width (5th segment); HL=head length; TL-HL=total length minus head length; PL=paramere length. Asterisks indicate significance at 5% (\*) or 1% (\*\*).

**Table 2.** Measurements of two *Quadraceps* species collected from two gull species

	<i>Q. punctatus</i> from <i>L. crassirostris</i> <i>n</i> =8 (male), 7 (female)	<i>Q. ornatus</i> from <i>L. schistisagus</i> <i>n</i> =7 (male), 9 (female)	P ( <i>t</i> -test)
PCHW-male	0.3513±0.0083	0.3843±0.0162	0.0002***
PCHW-female	0.3743±0.0053	0.4033±0.0180	0.0011**
THW-male	0.4688±0.0064	0.5223±0.0170	<0.0001***
THW-female	0.5014±0.0134	0.5655±0.0151	<0.0001***
MW-male	0.4375±0.0238	0.4657±0.0181	0.0240*
MW-female	0.4571±0.0281	0.4987±0.0348	0.0256*
AW-male	0.6338±0.0307	0.6600±0.0557	0.2700
AW-female	0.7214±0.0273	0.7267±0.0543	0.8198
HL-male	0.5550±0.0141	0.5400±0.0141	0.0612
HL-female	0.5829±0.0170	0.5756±0.0150	0.3793
TL-HL-male	1.5150±0.0507	1.4243±0.0959	0.0361*
TL-HL-female	1.8529±0.0531	1.7522±0.0736	0.0088**
GW-male	0.0899±0.0053	0.0850±0.0029	0.0462*
GW-female	0.0821±0.0057	0.0883±0.0056	0.0465*

Abbreviations: PCHW=precornal head width; THW=temporal head width; MW=metathoracic width; AW=abdominal width (5th segment); HL=head length; TL-HL=total length minus head length. Asterisks indicate significance at 5% (\*), 1% (\*\*) or 0.1% (\*\*\*)

**Fig. 1.** Habitus (left) and male genitalia (right) of (A) *Saemundssonina lari*, (B) *Quadriceps punctatus*, (C) *Quadriceps ornatus*. In the habitus figures, dorsal (left) and ventral (right) structures are shown. Arrows in (A) indicate measurements used for morphological analyses. Abbreviations: PCHW=precornal head width; THW=temporal head width; AW=abdominal width (5th segment); HL=head length; TL-HL=total length minus head length; PL=paramere length.

**Fig. 2.** Maximum likelihood tree of gull feather lice, *Saemundssonina lari*, *Quadriceps punctatus* and *Q. ornatus*. Some other species of *Saemundssonina* and *Quadriceps* were also included, and tree was rooted with *Ralicola*. Branch lengths are proportional to substitutions per site. Numbers associated with each branch indicate MP and ML bootstrap values. Circled numbers on *S. lari* indicate the haplotype ID mentioned in the text.

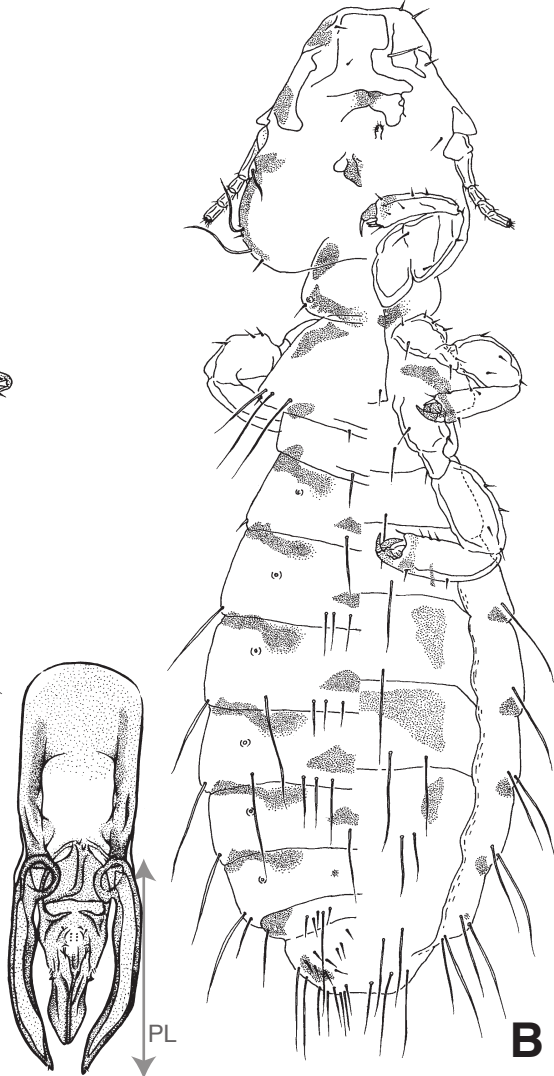
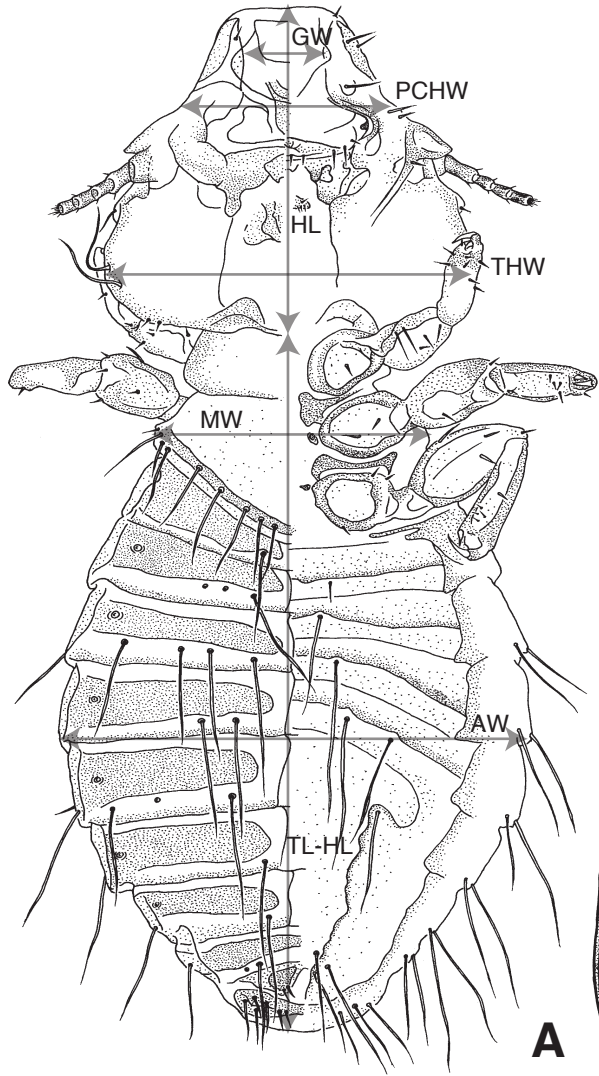
### **Online supplement**

Teuri.nex: next format text data of gene sequences

Saemundssonina.csv: csv data of morphological measurements of *Saemundssonina lari*

Quadriceps.csv: csv data of morphological measurements of *Quadriceps* spp.





○ ex. *Larus crassirostris*  
 ■ ex. *Larus schistisagus*  
 bootstrap values (MP/ML)

