Molecular identification of chewing lice and comparisons of host specificity between *Quadraceps* and *Saemundssonia* hosted by Charadriiformes

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Frontpage Photo: Adult *Saemundssonia lari* hanging on to feathers from common gull (*Larus canus*). Photo: Lena Kristiansen

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Abstract

Knowledge about chewing lice on gulls, auks, and waders (Charadriiformes) in Norway is scarce. The focus of this thesis was to collect and identify species from the two Ischnoceran louse genera *Saemundssonia* (Timmerman, 1936) and *Quadraceps* (Clay & Meinertzhagen, 1939) (Phthiraptera: Ischnocera) hosted by Charadriiformes in Norway. *Saemundssonia* is an ecomorphic "head-louse" with a body shape adapted to a life on the head and neck of the bird. *Quadraceps* is a typical ecomorphic "generalist" with a variety of body shapes living at different places on the body of the bird. In addition to morphological structures and host association, data from the mitochondrial (cytochrome c oxidase I) and nuclear (elongation factor-1α) markers were used for species identification.

I discuss whether differences in ecomorphs between the two closely related louse genera *Quadraceps* and *Saemundssonia* have an impact on host specificity of the louse. I also examined whether degree of host sociality may have an impact on the lice's degree of host specificity.

Saemundssonia lari was collected from six species of gulls from the genera *Larus* and *Chroicocephalus*, and *Saemundssonia celidoxa* was collected from two species of auks (*Alca torda* and *Uria aalge*). Among gulls, auks, and waders (Lari, Scolopaci and Charadrii) all *Quadraceps* species were restricted to one host species each. These findings suggest that *Saemundssonia* as an ecomorphic head-louse are less host specific than *Quadraceps* as an ecomorphic generalist-louse, supporting previous published findings. In this thesis, gregarious and colony breeding birds like, gulls and auks, are suggested to have more common louse species than more solitary birds like waders, but this pattern is less evident and needs further study.

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1 Introduction

Living close to nature, observation of birds has always been fascinating to me. Even more fascinating is the fact that birds are flying habitats hosting small ectoparasitic bird lice. Bird lice, commonly called chewing lice or featherlice, are hemimetabolous wingless insects living all their life stages in the plumage of birds. The lifetime of a bird is limited, so in order for next generations of chewing lice to survive they need to disperse to new birds. For this to happen, physical contact between bird individuals is important and chewing lice are therefore strongly associated with their host. Birds as hosts are analogous to islands and chewing lice can be transmitted to a novel host species in the same way as a free-living organism can disperse to a new island (Janzen 1968; Kuris *et al.* 1980; Koop *et al.* 2014). The distribution of birds is discontinuous and create transmission barriers for the chewing lice, but such barriers are not constant.

There are about 5000 known Phthiraptera-species present on roughly 4000 bird species, whereas Ischnocera is the largest suborder and contains about 3000 species (Smith *et al*. 2021). Phthiraptera are known from all bird orders (Price *et al.* 2003b) and no species of birds are known to lack lice entirely. Bird species houses on average three to five louse species each, whereas individual birds carry one to three louse species each (Gustafsson 2012). Phthiraptera are found to be paraphyletic (Murrell & Barker 2005), and Johnson *et al.* (2004) suggested the order to be polyphyletic. Phthiraptera have most probably evolved from free living ancestors belonging to the superorder Psocodea, which also gave rise to the Psocoptera (bark lice, book lice) (Lyal 1985; Johnson *et al*. 2004). The nearest living relatives of Phthiraptera are the Anoplura e.g., the human louse *Pediculus humanus* (Clay & Rothschild 1957). Chewing lice comprise the suborders Amblycera, Ischnocera, and Rhynchophthirina, but only Amblycera and Ischnocera are found on birds (and additionally parasitize mammals) (Price *et al.* 2003b).

This thesis focuses on the two genera *Saemundssonia* (Timmerman, 1936) and *Quadraceps* (Clay & Meinertzhagen, 1939), which both parasitize several groups of birds. *Saemundssonia* parasitize Anseriformes, Charadriiformes, Gruiformes, Pelecaniformes and Procellariiformes, and *Quadraceps* parasitize Charadriiformes and Ciconiiformes (Price *et al.* 2003b). Here I focus on *Saemundssonia* and *Quadraceps* species that are hosted by waders, gulls, and auks (order Charadriiformes), as both genera of lice are commonly found on all three suborders

(Lari, Scolopaci and Charadrii). Social birds more often house lice than more solitary ones, and they harbour more louse species, due to increased rate of horizontal louse transmission (Rózsa *et al*. 1996). Charadriiformes are therefore particularly interesting because the social interactions vary by species. According to Price *et al.* (2003b) each chewing louse species is found on an average of two bird taxa. Among Charadriiformes the number is 2.6 and somewhat higher, suggesting that a strict host-louse specificity seems to be the exception rather than the rule.

The suborder Ischnocera is monophyletic (Johnson & Whiting 2002; Barker *et al.* 2003), but many Ischnoceran genera are known to be paraphyletic (Johnson *et al.* 2002b). *Saemundssonia* and *Quadraceps* belong to the family Philopteridae (Price *et al.* 2003b) which contains nearly all Ischnoceran species from birds (Gustafsson *et al.* 2018). Philopteridae needs revision, but until a largescale family-level revision has been published, it is common to divide Philopteridae into a series of "complexes" in which *Quadraceps* and *Saemundssonia* belong to the "*Quadraceps*-complex" (Gustafsson *et al.* 2018).

Species identification of Ischnocera is challenging and there are few taxonomic experts. In addition, correct identification of the Ischnocera to species level is hampered by insufficient published species descriptions and partial or unsatisfactory illustrations (Gustafsson 2012). Identification of chewing lice has also too heavily relied on host classification and today identification of a new genus or species of lice solely based on louse-host interactions is not accepted (Johnson *et al.* 2002a; Price *et al.* 2003b; Hoberg & Brooks 2008). The *Quadraceps*-complex have not been revised completely and identification to species level is often in need to be done by comparisons with type material (Gustafsson *et al.* 2018). According to Hebert *et al.* (2003), four limitations regarding morphological species identification are recognized. First, phenotypic plasticity and genetic variability can lead to incorrect species identification. Second, morphologically crypsis can be overlooked. Third, morphological keys are often functional only for a specific life stage or gender, which complicates identification. Finally, the use of keys often demands high level of expertise leading to misdiagnosis. Animal species identification through DNA barcodes using the mitochondrial gene cytochrome c oxidase I (COI) was proposed by Hebert *et al.* (2003) as an approach to compensate the lack of taxonomic expertise. Generally, few studies have used molecular data to identify or discriminate Phthiraptera species. In this study, I used COI together with the nuclear gene elongation factor-1α (EF-1α), which has been identified as useful for studies of higher-level

phylogenetic relationship in insects (Danforth & Ji 1998). Because the two genes evolve at different rates (Johnson *et al.* 2003), combining both genes will increase the reliability of the results from the phylogenetic analyses.

When Ischnocera from different genera coexist on a bird, each is often restricted to a localized part of the hosts body (Askew 1971). As an adaption to escape behavior from bird preening, Ischnocera have developed different morphological features, termed ecomorphs, associated with specific ecological niches on the body of the bird on which they parasitize (Clay 1949; Johnson *et al.* 2005; Johnson *et al.* 2012) “Body lice” have short, rounded body, not greatly dorsoventrally flattened, with a broad bell-shaped head (Gustafsson *et al.* 2018). They live in the abdominal contour feathers avoiding preening by dropping between adjacent feathers or by burrowing into the downy portions of feathers (Clayton *et al.* 2016). “Head lice” live on the head and neck of the bird, out of reach from the birds preening. Head lice have a rounded body with a large triangular head to accommodate the large mandibular muscles in which they grip the barbs avoiding dislodging when the bird is scratching (Clay & Rothschild 1951¸ Askew 1971; Gustafsson *et al.* 2018). “Wing lice” are elongated and slender with long legs and escape from host preening by inserting themselves between the barbs of the wing or tail feathers (Yamagishi *et al.* 2014; Clayton *et al.* 2016; Gustafsson *et al.* 2018). Finally, lice that are “generalists” have no specific microhabitat preference on the bird body and have an intermediate body shape with a rounded head, but a variety of body shapes are described (Johnson *et al.* 2012; Clayton *et al.* 2016). They escape from preening by running quickly or escaping into the downy part of the basal feathers (Clayton *et al.* 2016). Species from the genus *Saemundssonia* (Figure 1) is regarded as an ecomorphic “head louse”. *Quadraceps* (Figure 2) is commonly regarded as an ecomorphic “generalist” (Gustafsson *et al.* 2018), but some *Quadraceps* species are also considered as an ecomorphic “wing louse” (Price *et al.* 2003b), most likely because they have the same form of body and exhibit the same escape behavior that other wing-lice use to avoid preening (Yamagishi *et al.* 2014).

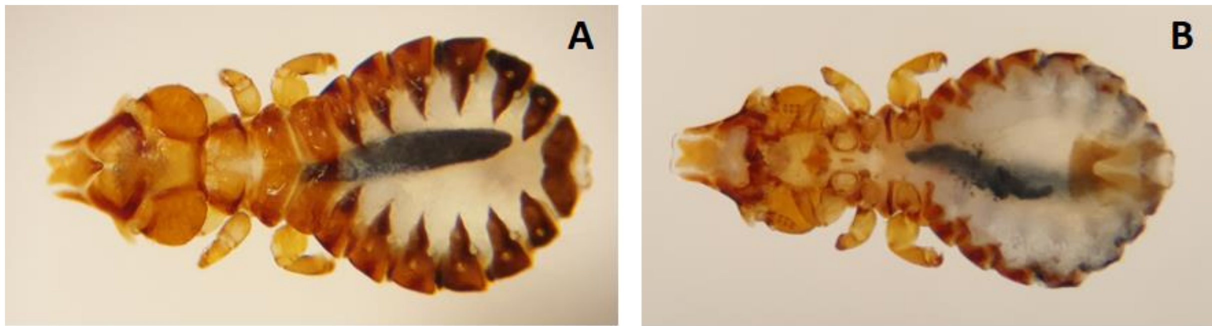

Figure 1. The ecomorphic "head-louse" *Saemundssonia celidoxa* (Burmeister, 1838) (Phthiraptera: Ischnocera) collected from a razorbill (*Alca torda*) **A)** dorsal view, **B)** ventral view. Photos: Lena Kristiansen.

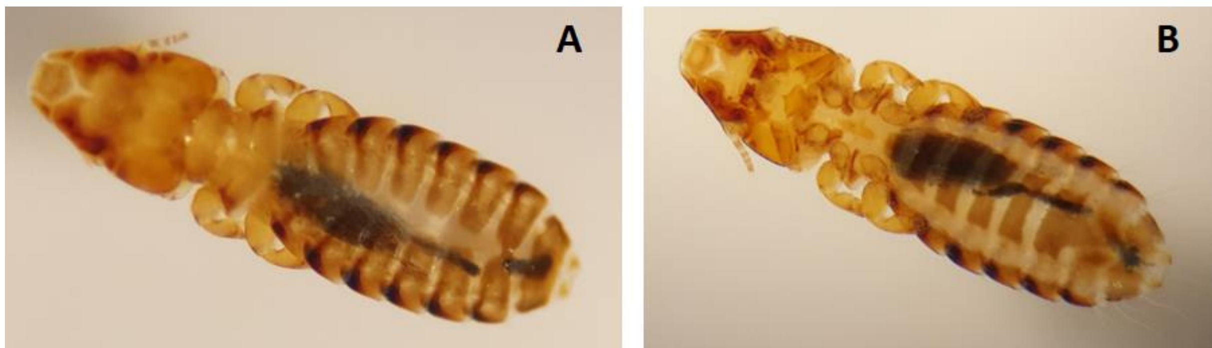

Figure 2. The ecomorphic "generalist-louse" *Quadraceps alcae* (Denny, 1842) (Phthiraptera: Ischnocera) collected from a razorbill (*Alca torda*). **A)** dorsal view, **B)** ventral view. Photos: Lena Kristiansen.

Available publications dealing with lice associated with birds from mainland Norway is scarce (Mehl 1981). Elven & Søli (2016) have estimated that only 7 % of the Phthiraptera species in Norway are known and tells that 24 Ischnoceran lice species have been reported, and approximately 350 Ischnocera species are yet to be found. In Norway five *Saemundssonia* species from Charadriiformes have been recorded (Mehl *et al.* 1982). This is *S. calva* (Kellogg, 1896) from thick-billed murre (*Uria lomvia*), *S. lari* (Fabricius [O.], 1780) from black-headed gull (*Chroicocephalus ridibundus*), lesser black-backed gull (*Larus fuscus*), glaucous gull (*Larus hyperboreus*) and black-legged kittiwake (*Rissa tridactyla*), *S. scolopacisphaeopodis* from Eurasian whimbrel (*Numenius phaeopus*), *S. tringae tringae* (Fabricius [O.], 1780) from purple sandpiper (*Calidris maritima*) and *S. tringae variabilis* (Denny, 1842) from dunlin (*Calidris alpina*). As far as I know, *Quadraceps* from Charadriiformes in Norway is only recorded from red knot (*Calidris canutus*) and identified to genus level (Mehl *et al.* 1982).

Aims

In this thesis I investigate the diversity and prevalence of *Saemundssonia* and *Quadraceps* hosted by Charadriiformes in Norway.

Important questions to be answered are:

- Does DNA barcodes support the species identifications based on morphology and host associations?

- Do the difference in use of microhabitat between the ecomorphic head-louse *Saemundssonia* and the generalist-louse *Quadraceps* have an impact on the degree of host specificity of the chewing lice?

- Are chewing lice more often less host specific among gregarious birds that tend to interact in mixed species flocks than more solitary ones that tend to interact in single species flocks?

1.1 Ischnocera

1.1.1 Morphology

Ischnocera are wingless and most species are dorsoventrally flattened (Mey 2002) (Figure 3), except head lice which have a swollen abdomen (Gustafsson *et al.* 2018). Their adult length overall varies and ranges between 2-4 mm (Mey 2002), females generally being bigger than males (Tryjanowski *et al.* 2007). The abdomen comprises of 11 variably sclerotized segments which constitutes the exoskeleton, but only 8-10 of these are visible (Price *et al.* 2003b). Ischnoceran lice are characterized by having fused meso- and metanotum, referred as the pteronotum, while the pronotum is separate (Price *et al.* 2003b; Gustafsson *et al.* 2018), (see Figure 12 and 13). They have no, or small vestigial eyes connected to the optic lobes by very thin optic nerves (Crespo & Vickers 2012). All three pair of legs are similar in size and shape with two claws on each tarsus, except that the first pair is directed forward rather than backward (Price *et al.* 2003b). Mouthparts (Figure 4) are mandibulate and the antennae are located laterally, consist of five segments, are large, filiform, and sexually dimorphic (Price *et al.* 2003b). Sometimes males have antennae modified as claspers for grasping the female during copulation (Clay & Rothschild 1957). Abdomen are covered with bristles or setae from the anterior margin of the head to the posterior margin of the body (Clay & Rothschild 1957).

Figure 3. Philopteridae sp. hanging on to the feathers from a common greenshank (*Tringa nebularia*) by using their legs. The body is wingless and dorsoventrally flattened. Photo: Lena Kristiansen.

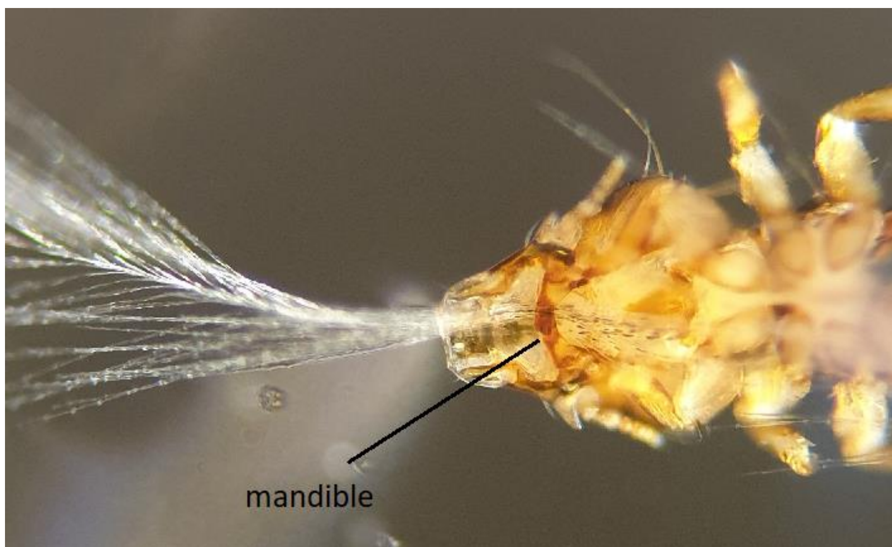

Figure 4. Ischnocera use mandibles to attach to feathers. Photo: Lena Kristiansen.

Pigmentation is generally the same on different parts of the louse body, but coloration vary between species (Gustafsson *et al.* 2018). Most lice are brown-beige-yellow (Gustafsson 2012), but coloration ranges from almost all white, to almost all black (Gustafsson *et al.* 2018). Dark-colored bird species tend to have dark-colored lice, whereas light-colored bird species tend to have light-colored lice (Rothschild and Clay 1957; Bush *et al.* 2010), but there is no evidence for crypsis among head louse which a bird can neither see nor preen (Bush *et*

al. 2010). Nymphs resemble less pigmented and smaller adults and are even more challenging to recognize to species (Figure 5).

Figure 5. Adult (left) and nymph (right) of *Saemundssonia lari* (Fabricius [O.], 1780), from common gull (*Larus canus*). Photo: Lena Kristiansen.

1.1.2 Biology

Chewing lice undergo three molt stages after the egg hatches (Clay & Rothschild 1957, Price *et al.* 2003b). Females produce 1-10 eggs per day which require 4-10 days to hatch, each nymphal stage requires 3-12 days, and adults live 1-2 months on their host (Clayton *et al.* 2016). Ischnoceran lice mainly feed on keratin-containing feathers which are digested with the aid of endosymbiotic bacteria (Møller & Rózsa 2005; Fukatsu *et al.* 2007). They may also feed on dead skin and skin waste (Clayton *et al.* 2008).

Chewing lice cannot live for long once outside their hosts (Askew 1971). Since chewing lice are wingless, they have developed different strategies for transmission to a new bird individual: i) vertical transmission from parents to offspring, ii) direct horizontal transmission with physical contact between hosts, or iii) indirect horizontal transmission without physical contact between hosts. Vertical transmission is by far the most common (Clay & Rothschild 1957) and may occur once the tips of the nestling`s feathers emerge (Clayton & Tompkins 1994). Direct horizontal transmission can occur during mating, between predator and prey, use of common dust bath and common roosting (Clay & Rothschild 1957; Timm 1983;

Hillgarth 1996). Feathers and nests are thought to facilitate indirect horizontal transmission of lice among species of birds (Timm 1983), but Ischnoceran lice are less likely able to crawl between nests in the wild due to low mobility when they are off feathers (Bartlow *et al.* 2016). Indirect horizontal transmission can also occur via phoresis (Clay & Rothschild 1957; Harbison *et al.*.2009; Bartlow *et al.* 2016), which is a short-lived association between two parasites in which the chewing louse attaches itself to a flying insect, which is less host specific than lice, solely for the purpose of transport (Corbet 1956). It has been suggested that as the body of a dead bird cools after death, the body of a fly may be more attractive or less repellent than the cooling flesh (Askew 1971). The least mobile louse species are the most phoretic and many species of Ischnoceran lice engage in phoresy extensively using their mandibles (Bartlow *et al.* 2016) and their third pair of leg (Harbison & Clayton 2011) to attach their flying means of transport.

In addition to lice, birds are commonly infested with other ectoparasites like fleas, feather mites, hippoboscid flies and mosquitos and have different behavioral and physiological strategies to control them (Clay & Rothschild 1957; Clayton & Tompkins 1994; Clayton *et al*. 2010). This includes preening and allopreening by the bill and scratching by the feet (Clayton *et al.* 2008). The relationship between bill morphology and louse abundance and diversity has been thoroughly studied (Barbosa 1996; Clayton & Walther 2001; Moyer *et al.* 2002) suggesting that bill shape (in addition to feeding) was selected for efficient preening (Moyer *et al.* 2002). Subtle differences in bill shape and size between bird species might therefore lead to different selective effects on chewing lice exploiting different hosts.

Harrison's rule states that the overall body size of most lice correlate with that of their hosts (Harrison 1915; Johnson *et al.* 2005; Cannon 2010). The ability of a louse to survive and reproduce on a novel host most likely depends on hosts being of similar size (Clayton *et al.* 2003; Bush & Clayton 2006) and closely related (Reed & Hafner 1997). Additionally, louse survival is thought to be low, due to difficulties in escaping host defences on a novel host (Clayton *et al.* 2003; Malenke *et al.* 2009). Tompkins & Clayton (1999) suggested that chewing lice require feathers with certain barb diameter for survival on a new host individual. Indeed, a match between hair width and claw size have been found in Phthiraptera gopher lice (Reed *et al.* 2000). In contrast, pigeon wing lice did not experience difficulties in remaining on feathers of different sizes (Bush *et al.* 2006).

1.2 Charadriiformes

The avian order Charadriiformes comprises three major suborders Lari, Scolopaci and Charadrii (Baker *et al.* 2007). Based on Baker *et al.* (2007), I have manually constructed a simplified phylogenetic tree for the birds included in my project, with branches representing bird genera (Figure 6). The separation of *Larus* and *Chroicocephalus* follows Pons *et al.* (2005) and the phylogenetic relationship for the suborder Scolopaci is confirmed by Gibson & Baker (2012).

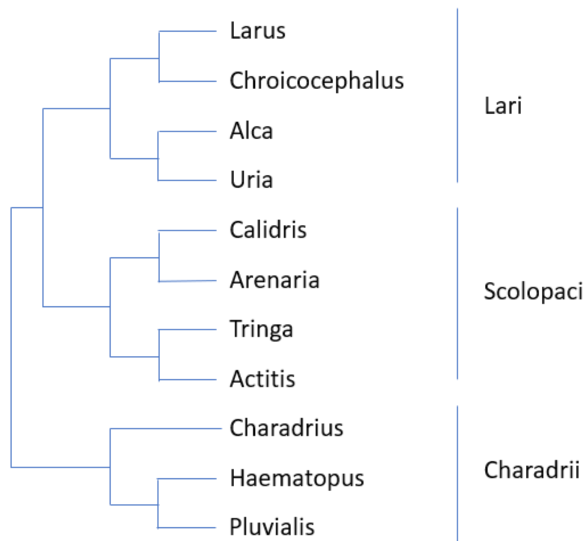

Figure 6. Simplified cladogram for included Charadriiformes genera belonging to the suborders Lari, Scolopaci and Charadrii based on Baker *et al.* (2007).

Few species of birds are solitary throughout all seasons of the year, as illustrated in Table 1. Many species in the suborder Lari breed colonially due to similar habitat preferences, reciprocal advantages in connection with predator protection and/or information exchange in association with food acquisition (Paludan 1960; Bergman *et al.* 1961; Cramp & Simmons 1983; Valle & Scarton 1999). Such colonies also attract waders (Valle & Scarton 1999). Several *Larus* species have overlapping habitats and large breeding distributions, and food-piracy and scavenging are common (Bergman *et al.* 1961; Cramp & Simmons 1983). Some gulls, like herring gull eat juvenile common guillemots and other auks, e.g. falling from breeding cliffs (Paludan 1960). Multispecies hybridization among Larus occurs frequently (Ottenburghs 2019) and several *Larus* species have propensity to hybridize (Liebers *et al.* 2004; Sonsthagen *et al.* 2016). Indeed,

lack of phylogenetic resolution has been found among *Larus* (Johnsen *et al.* 2010; Pons *et al.* 2014). Both razorbill (*Alca torda*) and common guillemot (*Uria aalge*) forms multi-species colonies and often mix on breeding cliffs (Cramp 1985). Both species might also feed in flocks (Cramp 1985). Additionally, both species are gregarious in the breeding season, but razorbill is less so in winter (Cramp 1985).

Scolopaci and Charadrii species mainly nest solitary, but many different species, genera and even families of different body sizes migrate, or roost together in flocks (Cramp & Simmons 1983; Gustafsson & Olsson 2017), (Table 1). *Tringa* species are either mainly solitary, like green sandpipers (*Tringa ochropus*), or weakly gregarious, like common redshanks (*Tringa totanus*), common greenshanks (*Tringa nebularia*), spotted redshanks (*Tringa erythropus*) and wood sandpipers (*Tringa glareola*) (Cramp & Simmons 1983). Green sandpipers can hybridize with common sandpipers (*Actitis hypoleucos*) (Cramp & Simmons 1983). Scolopaci and Charadrii have similar habitat- and food preferences, and a wide variety of social structures have been seen (Bancke *et al.* 1961; Cramp & Simmons 1983). For instance, ruddy turnstone (*Arenaria interpres*) is commonly seen together with other species of Charadrii throughout the year and will follow Oystercatchers (*Haematopus ostralegus*) to clean remains of discarded mussels. Ruddy turnstone might also nest among colonies of gulls (Laridae) and terns (Sternidae), and roost communally together with purple sandpiper and oystercatcher. Additionally, *Pluvialis* territories might overlap other *Charadrii* and *Charadrius* might associate with *Calidris* (Cramp & Simmons 1983).

In summary, all species of Lari, included in this thesis, breed colonially. Outside the breeding season most Lari species live close to other Lari species or mainly in mixed species flocks. Scolopaci and Charadrii species breed solitary, but degree of sociality and heterospecificity varies between species outside breeding season. During spring- and autumn migration many Scolopaci and Charadrii species tend to rest together in mixed species flocks at stopover areas where routes cross.

Table 1. Charadriiformes and their social abilities based on Cramp (1985) and Cramp & Simmons (1983).

Aves Suborder	Aves Species	Solitary Nesting	Colonially Nesting	Mainly Solitary Migration	Mainly Intraspesific Migration and/or Roosting	Mainly Mixed flock Migration and/or Roosting	Lekking system
Lari	*Chroicocephalus ridibundus* (black-headed gull)	X	X			X	
	Larus argentatus (European herring gull)	X	X			X	
	Larus marinus (great black-backed gull)	X	X			X	
	Larus fuscus (lesser black-backed gull)		X			X	
	Larus hyperboreus (glaucous gull)	X	X			X	
	Larus canus (common gull)	X	X			X	
	Alca torda (razorbill)		X	X			
	Uria aalge (common guillemot)		X	X			
Scolopaci	*Calidris canutus* (red knot)	X				X	
	Calidris pugnax (ruff)	X				X	X
	Arenaria interpres (ruddy turnstone)	X				X	
	Tringa erythropus (spotted redshank)	X				X	
	Tringa glareola (wood sandpiper)	X		X		X roosting	
	Tringa nebularia (common greenshank)	X				X	
	Tringa ochropus (green sandpiper)	X		X			
	Tringa totanus (common redshank)	X				X	
	Actitis hypoleucos (common sandpiper)	X			X		
Charadrii	*Charadrius hiaticula* (ringed plover)	X				X	
	Haematopus ostralegus (Oystercatcher)	X			X	X	
	Pluvialis apricaria (European golden plover)	X			X		

2 Materials and Methods

2.1 Collection of lice and fieldwork

The strategy was to obtain fresh louse samples from the birds in the order Charadriiformes. All lice specimens found on the inspected birds were collected, and the individuals not relevant for this study, i.e., not belonging to the two genera *Saemundssonia* and *Quadraceps,* were transferred directly to the DNA bank at the Natural History Museum in Oslo (NHMO).

Most of the lice were collected during 2020. Fresh louse samples were mainly collected from dead birds stored in freezers at the NHM. The bird individuals were found dead or shot due to airport security during the last 13 years. Fresh louse samples were also collected from common guillemots and razorbills, found dead in Oslofjorden in Oslo November 2020. Additionally, lice collected from living birds by bird ringers at different bird-ringing stations, e.g., Jomfruland and Revtangen, were included in the study.

Fieldwork was carried out in June 2020 at the NHMs field station in Øvre Heimdalen, Innlandet, where four great snipes (*Gallinago media*) were captured and investigated. Additionally, one common snipe (*Gallinago gallinago*) nest was investigated. We tried but failed catching any common snipe. Another fieldtrip was carried out in July 2020 along the river Mysenelva, Viken, where one common sandpiper was captured and investigated for lice. No lice were found, neither on the great snipes, the common sandpiper nor in the nest. Additionally, I have used eight fresh lice from the insect collection at NHMO provided by bird ringers to the project "Featherlice in Norway". Five old louse samples, collected from living birds in Finnmark in 1980 and 1981 and stored in tubes with low ethanol content and at room temperature, have been tested for DNA content.

2.1.1. Sampling of lice from dead and living bird specimens

<u>Sampling of lice from dead birds</u>

Fingers, tweezers, small brush and a hand magnifier or magnifying glass were used to manually look for lice. A white bench surface made it easier to detect any lice that had fallen off the bird during the search. Good lighting was important when looking for small, sometimes camouflaged lice (Figures 7 and 8).

Figure 7. Searching for feather lice on a dead juvenile western curlew (*Numenius arquata*) using good lighting and different equipment. Photo: Lena Kristiansen.

Before starting, birds from the freezer were kept at room temperature for a few hours, sometimes overnight. Head, wings, and body of the birds were systematically and thoroughly investigated. An extra look around the eyes and bill were made. Finally, the skin was shaken or patted. Finding lice when the plumage of the bird was wet could be a challenge. In some cases, a hair dryer was used. To avoid contamination, hands and bench were washed thoroughly between handling of each bird individual. Individual birds were kept separate but, in some cases, it is uncertain whether the bird collector did this before the birds were placed separately in the freezer for storage. 12 common guillemots and one razorbill found dead in Oslofjorden in Oslo November 2020 were put in a common plastic bag by the collector.

Figure 8. Searching for lice on a dead juvenile western curlew (*Numenius arquata*). Photo: Lena Kristiansen.

Sampling of lice from living birds

During fieldwork, we caught four great snipes and one common sandpiper. Birds were captured with mist nets in collaboration with experienced bird ringers (Figure 9). When looking for lice on living birds, one person held the bird, while another was searching systematically for lice using fingers and tweezers. At dusk when we were looking for lice on great snipes in Heimdalen with no access to electricity, external light sources as headlight and candles were used (Figures 10 and 11). No lice were found, and birds therefore not listed in the table.

Figure 9. Using mist net in the habitat of common sandpiper (*Actitis hypoleucos*) along the river Mysenelva in Viken. Photo: Gaute Grønstøl (NHMO).

Figure 10. Two working together searching for lice on a common sandpiper (*Actitis hypoleucos*) using tweezers and fingers. Photo: Gaute Grønstøl (NHMO).

Figure 11. Searching for lice on great snipes (*Gallinago media*) during the fieldwork in Heimdalen, using tweezers and fingers. Photo to the left: Gaute Grønstøl (NHMO). Photo to the right: Silje Larsen Rekdal (NHMO).

<u>Searching for lice on eggs and nest</u>

During fieldwork in Heimdalen, we also looked for nests. One nest with four eggs belonging to a common snipe were investigated. Eggs were carefully lifted, nest and eggs examined with the naked eye and put back in the same position. The nest was carefully touched, and we worked as quickly as possible to avoid the bird from rejecting the nest. No lice were found, and therefore not listed in the table.

2.1.2 Evaluation of contamination risk

Both when collecting lice from living or dead birds there is a risk of contamination, because lice specimens can be transferred from one bird individual to another by us and the methods we use. Awareness of contamination risk is important because it can affect the results, in particular when it comes to determining louse-host interactions.

Ischnocera lice have bristles and powerful mandibles ensuring that they are well anchored to the plumage, and do not actively leave their host if it dies (Mey 2002). Even so, lice can fall off or move out of the plumage during handling of both dead and living birds. Due to contamination risk, it is important to put both living and dead birds in separate containers or bags during collection/capture, storage, and handling. In addition, efficient cleaning of hands, bench, and other equipment, between handling of bird individuals is crucial to prevent the transmission of lice between bird individuals.

During the collection of lice from dead birds, it was challenging to evaluate contamination risk when collection and storage conditions were not documented. Collections of dead birds had been done by people with varying knowledge of what the birds should be used for. I experienced that 12 newly dead common guillemots, and one razorbill collected in Oslofjorden 2021 were delivered to the museum in a common sack. Lice were still alive and borrowed down in the plumage. Due to contamination risk, examination of live or freshly collected birds are preferred.

2.1.3 Morphological identification of louse individuals

After collecting the lice, each sample (containing several individuals) was investigated using a microscope (WILD HEERBRUGG Switzerland M5-96583). Firstly, I separated Ischnoceran lice from Amblyceran lice based on external louse morphology like body shape, head, and antennae, as illustrated in figure 12. Secondly, I selected lice from the two genera *Saemundssonia* and *Quadraceps* (Ischnocera: Philopteridae). To make suborder, family, and genus identification of the Ischnocera lice, I used external morphological character descriptions made by Gustafsson *et al.* (2018), Johnson *et al.* (2012), Mateo (2006) and Price *et al.* (2003b). Illustrations of the general morphology of the body, head, and male genitalia among *Saemundssonia* and *Quadraceps*, were found in Mateo (1988). The dorsal view of preantennal structures in Philopteridae were found in Johnson *et al.* (2012). Species determination was challenging. As a basis, I used information about host-louse interactions already known for science, as presented in Table I in the Appendix, using sources like Price *et al.* (2003b), Gustafsson *et al.* (2018), and Smith *et al.* (2021).

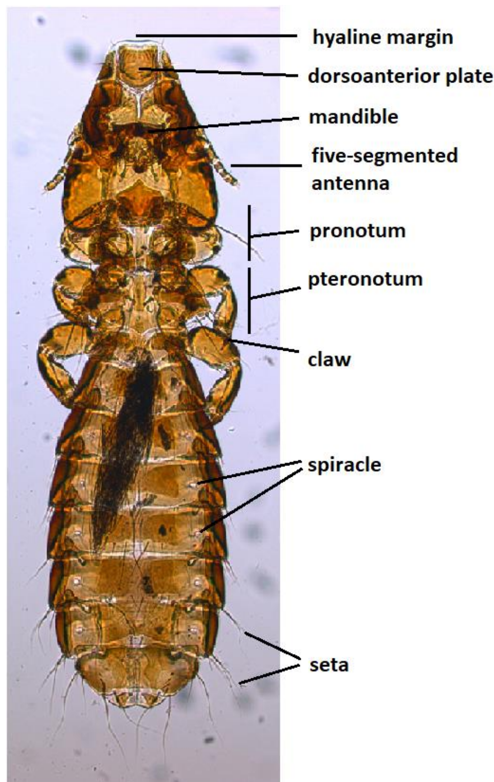

Figure 12. General external morphological characters in Ischnocera, represented by the louse species *Quadraceps obliquus* from common guillemot (*Uria aalge*). Terminology follows Price *et al.* (2003). Photo: Lena Kristiansen.

2.2 Laboratory work

2.2.1 Preparation of louse-samples

Lice are covered by a sclerotized exoskeleton, which is important for morphological identification. Therefore, after DNA-extraction it is desirable to preserve the louse exoskeletons and use them as a reference collection for morphological identification stored at NHM and as vouchers for the DNA data. During DNA-extraction it was therefore desirable to treat the specimen with caution in order not to destroy the exoskeleton.

Fresh samples

To extract as much DNA as possible and preserve intact exoskeleton, I tested different methods to prepare the louse before performing the DNA-extraction. I performed DNA-extraction both with intact exoskeleton and after perforating the exoskeleton (see 2.2.2). the place of perforation was the same as in Gustafsson *et al.* (2018) and was mainly made half-

way through the body dorsally between pronotum and pteronotum (Figure 13), but also at a random place at the abdomen before becoming more trained or when louse individuals was very slim. I selected mainly adult louse individuals because they are easier to identify. Adult lice are somewhat bigger than nymphs and therefore easier to perforate and might yield higher contents of DNA.

Different tools to perforate the exoskeleton were tested; a) Stainless-Steel Insect pin - size 0. This Insect pin is 0.35 mm thick and has a length of 38 mm, which enables a good finger grip. b) Micro-Needle and c) Microlet Lancets (Bayer). Between each perforating, the tools were sterilized or replaced. Sterilization was done by dipping the perforating tool in 95% ethanol followed by burning with open flame.

As a result, after DNA extraction, five of seven lice with intact exoskeleton and one of 11 lice with perforated exoskeleton had too low values to be measured by Invitrogen ™ Qubit® 2.0 Fluorometer. Based on these findings I experienced that perforation of louse exoskeleton before DNA-extraction led to higher probability of measurable DNA content in the sample compared to samples where the exoskeleton remained intact. Both using tools like Insect pin – Size 0, Micro-Needle and Microlet Lancets made it easier to extract DNA, but Insect pin were easier to sterilize and handle for accuracy. I therefore continued to use Insect pin.

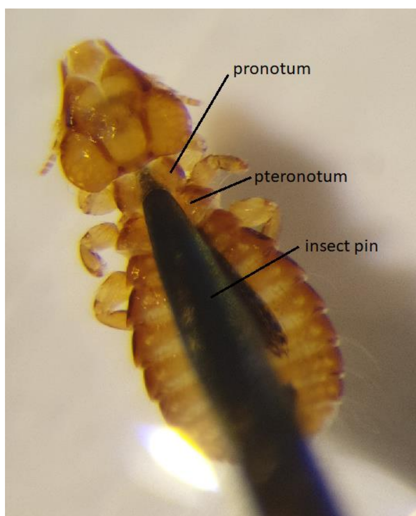

Figure 13. Perforation of louse exoskeleton dorsally between pronotum and pteronotum with a Stainless-Steel Insect pin - Size 0. Photo: Lena Kristiansen.

Old samples from the 1980s

Several louse samples, collected by Jan Terje Lifjeld in 1980 and 1981, had been stored in tubes with low ethanol content in room temperature for many years, which are factors that influence the amount and fragmentation of the DNA. To test if I would still be able to extract sufficient amount of DNA and with satisfactory quality, I included five different louse-host interactions in the study: *Saemundssonia platygaster platygaster* and *Quadraceps hiaticulae/fissus* from common ringed plover (*Charadrius hiaticula*), *Saemundssonia lari* from black-legged kittiwake (*Rissa tridactyla*), *Saemundssonia tringae* from dunlin (*Calidris alpina*), and *Quadraceps obtusus* from common ringed plover (*Charadrius hiaticula*). To improve the chances to obtain the DNA from these samples, I decided to pool three individuals from the same host individual in one DNA-extraction sample. A total of 15 old louse individuals, were therefore used.

2.2.2 DNA extraction

Total genomic DNA was extracted from 125 louse individuals using the E.Z.N.A. ® Tissue DNA Kit according to the protocol of the manufacturer (Omega Bio-Tek), with some exceptions or adjustments (described below). Before each handling of fresh louse individuals, tweezers were disinfected using chlorine bath, 96% ethanol and finally burned with open flame. Qubit ™ dsDNA HS (High Sensitivity) Assay Kit was used for DNA quantitation following the protocol of the manufacturer (Invitrogen™). Standards and samples were read by Qubit® 2.0 Fluorometer (ThermoFisher Scientific).

Lice were selected based on morphological species identity, and louse-host interactions included outgroup for use in phylogenetic trees. If possible, adult individuals were chosen over nymphs. Some lice were also selected to test PCR program and perforating method. I used relatively high number of lice samples to increase the likelihood of a successful outcome through the whole process, from a single louse to a DNA-sequence of good quality, from as many louse-host interactions as possible.

E.Z.N.A. ® Tissue DNA Kit

Fresh louse individuals (n = 125) were separately placed in 1.5 mL microcentrifuge tubes and thereafter centrifuged. Louse individuals were dried in a heating cabinet, with the lid of the tubes left open, between 65-70 °C for 2-5 minutes based on the size of the louse or in DNA120 Speed Vac ® (Thermo Savant) at medium drying rate for 3 minutes. Old louse samples (= 15 specimens from 5 species) from the 1980s were treated differently before reagents were added; three whole and dried louse-individuals from each louse-species were grinded with a sterilized pestle and tissue transferred to a separate 1.5 mL microcentrifuge tube, representing each sample.

I added 200 µL TL Buffer and 25 µL Proteinase K Solution to each louse/tissue containing tube and vortexed thoroughly. After centrifugation the sediment was incubated at 55 °C, 250 rpm on a heating block overnight. After incubation, the samples were centrifuged to remove condensation. Louse exoskeletons were picked up from the microcentrifuge tubes, separately washed in purified water (Milli-Q) and transferred to 96% ethanol-containing tubes for later and further preparation. Grinded tissues were centrifuged at maximum speed (≥10,000 x g) for 5 minutes and transferred to a sterile 1.5 mL microcentrifuge tube without disturbing or transferring any of the insoluble pellet. 220 µL BL Buffer were added all samples and vortexed. After incubation at 70 °C for 10 minutes in a heating cabinet, 220 µL 100% ethanol were added and vortexed. The entire sample (included any precipitates) were transferred to a provided HiBind®DNA Mini Column inserted in a 2 mL Collection Tube and centrifuged at maximum speed for 1 minute. The filtrate was discarded, and the collection tube reused. 500 µL HBC Buffer diluted with 100% isopropanol were added and centrifuged at maximum speed for 1 minute. The filtrate and collection tube were discarded. The HiBind®DNA Mini Column was inserted to a new provided 2 mL Collection Tube. 700 µL DNA Wash Buffer diluted with 100% ethanol was added and centrifugated at maximum speed for 1 minute. Filtrate was discarded and collection tube reused. The wash step with 700 µL DNA Wash Buffer was repeated and filtrate discarded. The empty HiBind®DNA Mini Column was centrifuged at maximum speed for 2 minutes to dry the column and transferred to a nuclease-free 1.5 mL microcentrifuge tube. 50 µL Elution Buffer heated to 50 °C for 5 minutes was added and centrifuged at maximum speed for 1 minute. Sample was heated in a heating cabinet at 50 °C for 5 minutes. Adding of 50 µL Elution Buffer was repeated for a second elution step, centrifuged at maximum speed for 1 minute and placed in a heating cabinet at 50

°C for 5 minutes. Eluted DNA samples were stored in freezers at 20°C for later and further analyses.

Qubit®dsDNA HS Assay Kit

All reagents were at room temperature. The required numbers of clear thin-walled 0.5 mL PCR® Tubes (Axygen®PCR-05-C tubes) for 2 standards (Standard #1 and Standard #2) and selected DNA extracted louse-samples (n) were set up. Qubit® working solution was prepared by diluting the Qubit® dsDNA HS Reagent 1:200 in Qubit® dsDNA HS Buffer in a clean 5 mL plastic tube. 10 µL from each standard and 2 µL from each louse sample were added to separate PCR tubes. 190 µL of Qubit working solution were added the 2 standard tubes and 198 µL Qubit working solution were added to each louse-sample (n). Standards and samples were vortexed 2-3 seconds followed by spinning. All tubes were incubated at room temperature for 2 minutes. Concentrations of the DNA extracts were measured using Invitrogen TM Qubit® 2.0 Fluorometer (ThermoFisher Scientific) either the same day as or after the DNA extracts were made.

The amount of extracted DNA varied greatly across samples. Among fresh samples the concentrations ranged between 0.055 ng/µl and 5.750 ng/µl, with an average of 0.360 ng/µl (if samples with too low values were excluded). 10 of 113 lice with perforated exoskeleton had due to TM Qubit® 2.0 Fluorometer, too low DNA content to be measured and excluded for further procedures. Two of the five old samples had too low DNA content to be measured. The concentrations for the remaining three samples were 0.100 ng/µl, 0.105 ng/µl and 0.227 ng/µl. Despite lack of results on TM Qubit® 2.0 Fluorometer, all five samples were selected for further procedures.

2.2.3 Polymerase Chain Reaction (PCR)

For further analyses, I picked out 62 lice samples belonging to the two genera *Saemundssonia* and *Quadraceps* based on morphological species identity, the highest DNA content registered by the Qubit® 2.0 Fluorometer and host-louse interaction. To increase the probability for successful DNA-amplifying from as many louse species and louse-host interactions as possible, I amplified three to four samples from each host-louse interaction or less when this

was not possible. Additionally, I tried to amplify DNA from five old louse samples from the 1980s. These old samples had either a host-louse interaction not already collected by me or a host-louse interaction that I had only one more sample of. Selected working solutions were stored in refrigerator during the period when PCRs were made.

All samples were amplified for the regular mitochondrial marker, COI, using the primers LepF1 and LepR1 (Hebert *et al*. 2018; Hernándes-Triana *et al.* 2014) and one nuclear marker, EF-1α, using the primers EF1-For3 and Cho 10 (Danforth & Ji 1998), presented in Table 2 below. Additionally, I tested the nuclear markers hypothetical protein (Hyp) and transmembrane emp24 domain-containing protein 6 precursor (TMEDE6), for seven samples each by using the primer pairs described by Sweet *et al.* (2014). PCR reactions were set up in a total volume of 12.5 µl, including 10,5 µl of master mix (1.25 µl buffer, 1.25 µl $MgCl_2$, 0.6 µl dNTP, 0.6 µl BSA, 6.1 µl dH_2O, 0.3 µl of each primer, 0.1 µl AmpliTaq and 2 µl of DNA extract). Reaction products were run on 1% agarose gels at 90 V for 40 minutes.

Table 2. Forward and reverse primers used for obtaining partial sequences of the elongation factor 1 alpha (EF-1α) and cytochrome c oxidase subunit I (COI).

Locus	Primer name and sequence (5´- 3´)		Source
CO1	Forward	LepF1: ATTCAACCAATCATAAAGATATTGG	Hebert *et al.* (2018) and
	Reverse	LepR1: TAAACTTCTGGATGTCCAAAAAATCA	Hernándes-Triana *et al.* (2014)
EF-1α	Forward	EF1-For3: GGNGACAAYGTTGGYTTCAACG	Danforth & Ji (1998)
	Reverse	Cho10: ACGGCVACKGTYTGHCKCATGTC	

I evaluated the success of each PCR reaction according to the brightness and clarity of the bands on agarose gels. Based on these results I selected the two best amplifying loci; COI and EF-1α and did not continue with Hyp and TMEDE6. Poorly performing samples using COI and EF-1α, were optimized in different ways. I tested different annealing temperatures and number of cycles and doubled the amount of DNA extract. Some samples with primer-dimers were run with less amount primers. For COI, I also tested step-down annealing temperatures for samples with no or low success and nested PCR for samples with no results on the gel. Step-down and nested PCR program generally did not give any results on the gel. Optimized PCR programs used for amplification of COI and EF-1α are presented in Tables 3 and 4.

Table 3. Optimized PCR programs used in the amplification of the mitochondrial locus cytochrome c oxidase subunit I (COI) region. Program 2 was used when Program 1 had no or low success.

	Program 1 (CO1)	**Program 2 (CO1)**
Denaturation	94℃ - 1 min	94℃ - 1 min
Amplification	94℃ - 40 s, 51℃ - 40 s, 72℃ - 1 min (x35)	94℃ - 40 s, 56℃ - 40 s, 72℃ - 1 min (x30)
Extension	72 ℃ - 5 min	72 ℃ - 5 min
Hold ∞	10℃	10℃

Table 4. Optimized PCR program used in the amplification of the nuclear locus elongation factor 1 alpha (EF-1α) region.

	Program (EF-1α)
Denaturation	94℃ - 2 min
Amplification	94℃ - 40 s, 55℃ - 30 s, 72℃ - 1 min (x32/38)
Extension	72 ℃ - 4 min
Hold ∞	10℃

Old samples

DNA quality recovered from older insect specimens is frequently degraded dependent on preservation method, storage conditions and the amount of time since specimens were collected (Watts *et al.* 2007; Lalonde *et al.* 2020). Samples stored away from ultraviolet radiation at low temperatures, are likely to suffer less nucleic acid degradation (Hofreiter *et al.* 2001; Watts *et al.* 2007). Insects killed and stored in 100% ethanol, kept in the fridge below 4°C have shown to give good amount and/or quality of DNA extraction (Dillon *et al.* 1996). A general decline in PCR success, either as the number of successful PCRs or the length of the fragment that may be amplified, increases with specimens age (Watts *et al.* 2007). None of the old samples contained DNA that could be amplified by PCR, and they were thus excluded from further genetic analysis. Right combination of techniques for DNA extractions, primer design and amplification can make it possible to obtain DNA from old samples (Lalonde *et al.* 2020), but due to time constraints of this thesis, I chose not to test this further.

2.2.4 Making PCR products ready for DNA Sequencing

Purified samples were sent off to sequencing through the commercial services of Macrogen Europe B.V. (Netherlands). I made PCR-products ready following manual details about dilution and primer concentration made by the company. First, I wanted to get rid of unused primers and unused dNTPs in my PCR products before sequencing with an enzyme mix. IT/Exostar were diluted 10 times (20 µl Exosap-IT + 180 µl dH_2O for 96 samples). I had 144 samples, distributed on three plates. 2 µl diluted Exosap-IT were added to each well. All PCR products were spun and 6 µl transferred to the designated well according to my cleaning log table. The plates were vortexed and spun for 2 minutes at 850 rpm in a centrifuge, incubated at 37°C for 45 minutes followed by 80°C for 15 minutes and thereafter spun again. I added 30 µl dH_2O to samples with extraordinarily strong PCR bands and 10 µl dH_2O to the other samples followed by vortex and spin. Primers and cleaned PCR products were mixed in new, labeled plates. I transferred 7,5 µl cleaned PCR product to separate wells and added 2,5 µl primer. The plates were sealed and sent to Macrogen Europe B.V. In total, 288 sequences were received from Macrogen Europe B.V., representing 144 PCR products. Some sequences represent the same louse individual to ensure or increase the probability of obtaining sequences from as many louse-host interactions as possible.

2.2.5 Sequence Alignment and Phylogenetic Analyses

After receiving sequences from Macrogen Europe B.V., I used the program CodonCode Aligner 9.0.1. to analyze and troubleshoot the Sanger Sequencing results by evaluating the chromatograms with respect to the peak pattern. Subsequently, the sequences were manually cut at appropriate sites and the forward and reverse sequences combined. Sequences of bad quality were excluded. I selected sequences representing different louse individuals and louse-host interactions. In total, 95 PCR products had good quality regarding both strands, 34 PCR products had good quality regard one strand, and 15 PCR products were excluded. In addition, I included 34 sequences of interest based on COI from the project "Featherlice in Norway", project name NORLI in Barcode of Life Data System (BOLD). Four sequences were also found in GenBank and BOLD, but generally there were very few sequences available from chewing lice species.

I generated and compared multiple louse gene trees using the Molecular Evolutionary Genetics Analysis Version 7.0 (MEGA7) software program described by Kumar *et al.* (2016), using the Neighbor Joining method (Saitou *et al.* 1987; Tamura *et al.* 2004). I set numbers of bootstrap iterations to 1000, substitution type as nucleotide and the model as Kimura 2-parameter, substitution to including transitions and transversions, rates among sites to be uniform, gaps/missing data are deleted and all codon positions were included. Separate gene trees of *Saemundssonia* species and *Quadraceps* species were constructed based on EF-1α and COI (Figures 16-19). As a phylogenetic outgroup I selected *Goniodes sp.* found in the plumage of a dead ruff. In addition, two separate COI and EF-1α gene trees were constructed including both suborders (Figures 14 and 15).

2.3 Registration and mounting

All chewing louse samples received their own unique code throughout the process from the time a louse was picked from its host to the finished DNA sequence, presentation in gene trees or slide mounting. Lice sampled by me were marked with an "L", and lice sampled by bird ringers with an "S", followed by a number and most often a letter. The number indicated one specific bird individual, and the letter indicated one specific louse individual picked from this specific bird individual. For example, "L-2A" and "L-2B" indicated that two lice, "A" and "B", were collected by me from the same bird individual "L-2". Louse samples from the project "Featherlice in Norway" was labeled as "NORLI", sequences from BOLD were labelled as "NHMO-DAR". This code system provided a good overview of which louse was found on its respective host. All newly generated DNA sequences were deposited in GenBank and BOLD.

After DNA-extraction, selected louse exoskeletons were mounted on microscope slides using Euparal. All slides were labeled manually with collector name, date, code, louse species or genus and host species. Photos were made with a microscope camera Leica DFC420 mounted on a microscope Leica DM6000B, using the software program Leica Application Suite (LAS) version 4.13.0.

2.4 Ethical considerations

I mainly examined dead birds stored in freezers. These birds had either been found dead or shot as safety measure on Gardermoen airport. Live birds were captured with mist nets guided by and/or done by experienced and approved bird ringers. Nest and eggs were investigated fast and carefully, after which the area was left quickly. Permission to capture and handle birds was given to Arild Johnsen by Mattilsynet (ID23294). I have completed the course in experimental animal science for researchers, as required in the Norwegian Regulation on Animal Experiment, § 24 and Annex E, Points 1-11. There is no reason to believe that the birds suffered during fieldwork.

3 Results

Altogether, 125 louse samples were included in the phylogenetic analyses (Figures 18-21), either based on sequences from EF-1α (52 samples), COI (73 samples), or both markers (34 samples) (Table II in the Appendix). This included molecular data from 14 species in the genus Quadraceps and five Saemundssonia species. Analysis of deeper phylogeny of the two genera was not a part of this thesis, but some general patterns were identified. The COI gene tree combining the two genera revealed that Saemundssonia was monophyletic with a bootstrap value of 99, whereas Quadraceps was divided in several groups with bootstrap values < 90 (Figure 14). In the combined EF-1α gene tree, Quadraceps and Saemundssonia were paraphyletic (Figure 15).

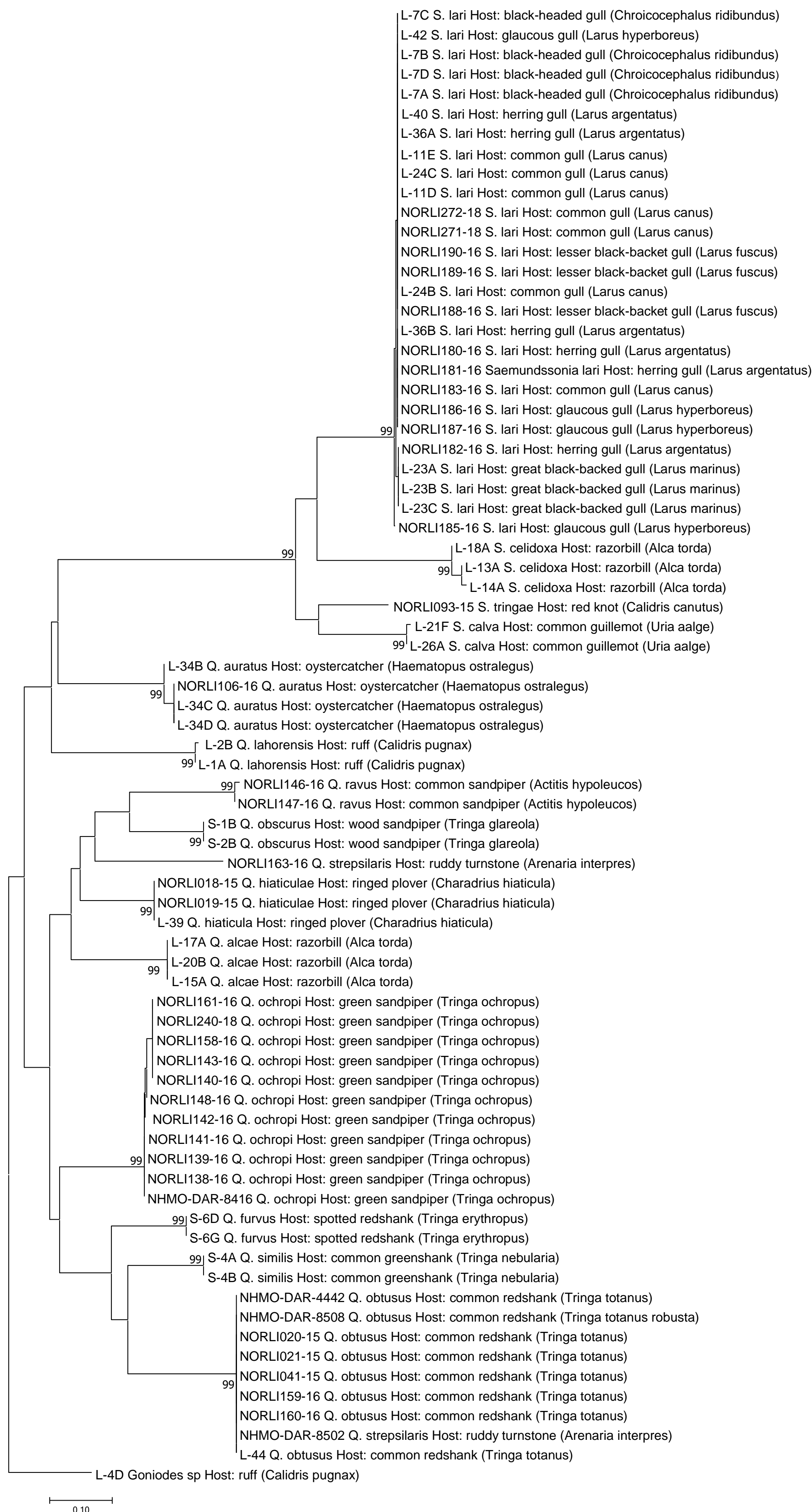

Figure 14. Neighbor-Joining tree for *Quadraceps* and *Saemundssonia* species based on sequences from the mitochondrial marker COI. Numbers and letters before louse-host species name indicate code for this specific interaction. Numbers at nodes indicate bootstrap values from 1000 replicates. Bootstrap values below 90 were excluded. Phylogenetic outgroup is represented by *Goniodes sp.* (L-4D).

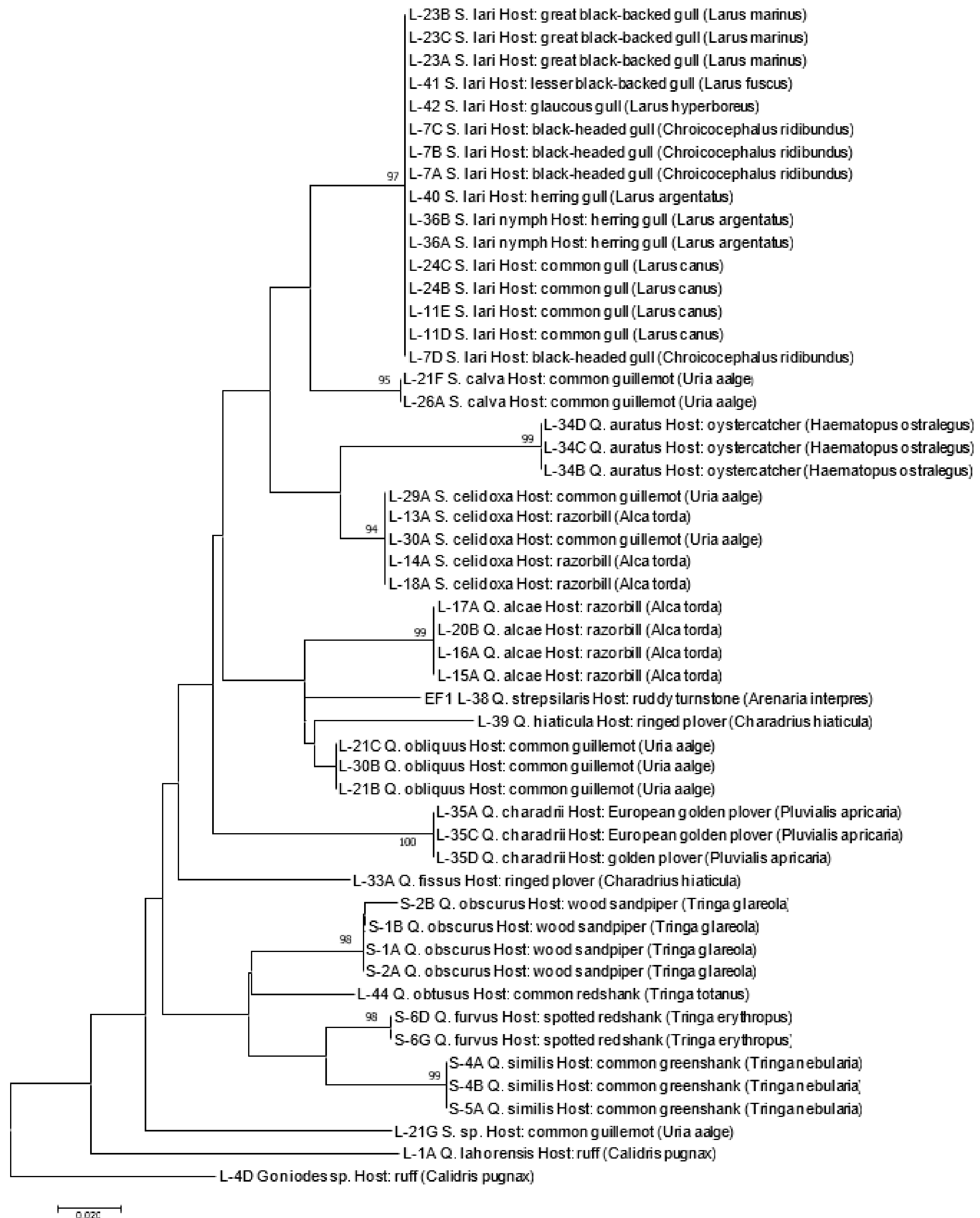

Figure 15. Neighbor-Joining tree for *Quadraceps* and *Saemundssonia* species based on sequences from the nuclear marker EF-1α. Numbers and letters before louse-host species name indicate code for this specific interaction. Numbers at nodes indicate bootstrap values from 1000 replicates. Bootstrap values below 90 were excluded. Phylogenetic outgroup is represented by *Goniodes sp.* (L-4D).

3.1 Identification

Most specimens identified based on morphology and known louse-host interactions made up clearly defined clades, supported by both molecular markers. The species represented by more than one sequence in the gene trees, had good stastitical support, both regarding COI (bootstrap values of 100) (Figures 16 and 18) and EF-1α (bootstrap values > 93) (Figures 17 and 19). Generally, the basal branches of the gene trees have low bootstrap values, except *Q. furvus* and *Q. similis* (Figure 18).

Identification of nymphs is known to be challenging. Two nymphal specimens collected from herring gull (L-36A and L-36B) were identified as *S. lari* based on morphology and recorded louse-host interactions. The nymphs were supported in both COI and EF-1α genes, as the sequences cluster together with other *S. lari* in the gene trees (Figures 16 and 17).

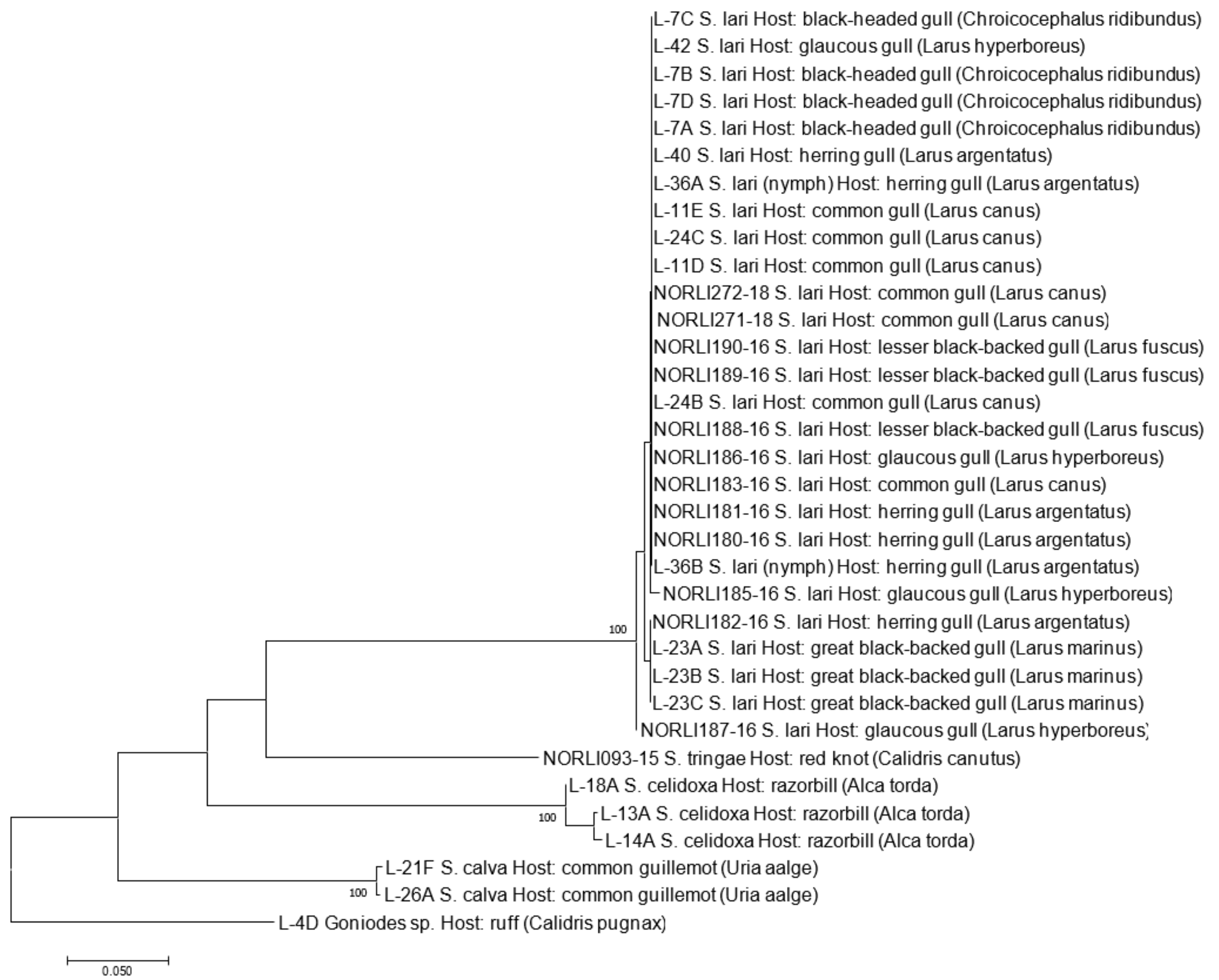

Figure 16. Neighbor-Joining tree for *Saemundssonia* species and their hosts based on sequences from the mitochondrial marker COI. Numbers and letters before louse-host species name indicate code for this specific interaction. Numbers at nodes indicate bootstrap support. Bootstrap values below 90 were excluded. Phylogenetic outgroup is represented by *Goniodes sp.(*L-4D).

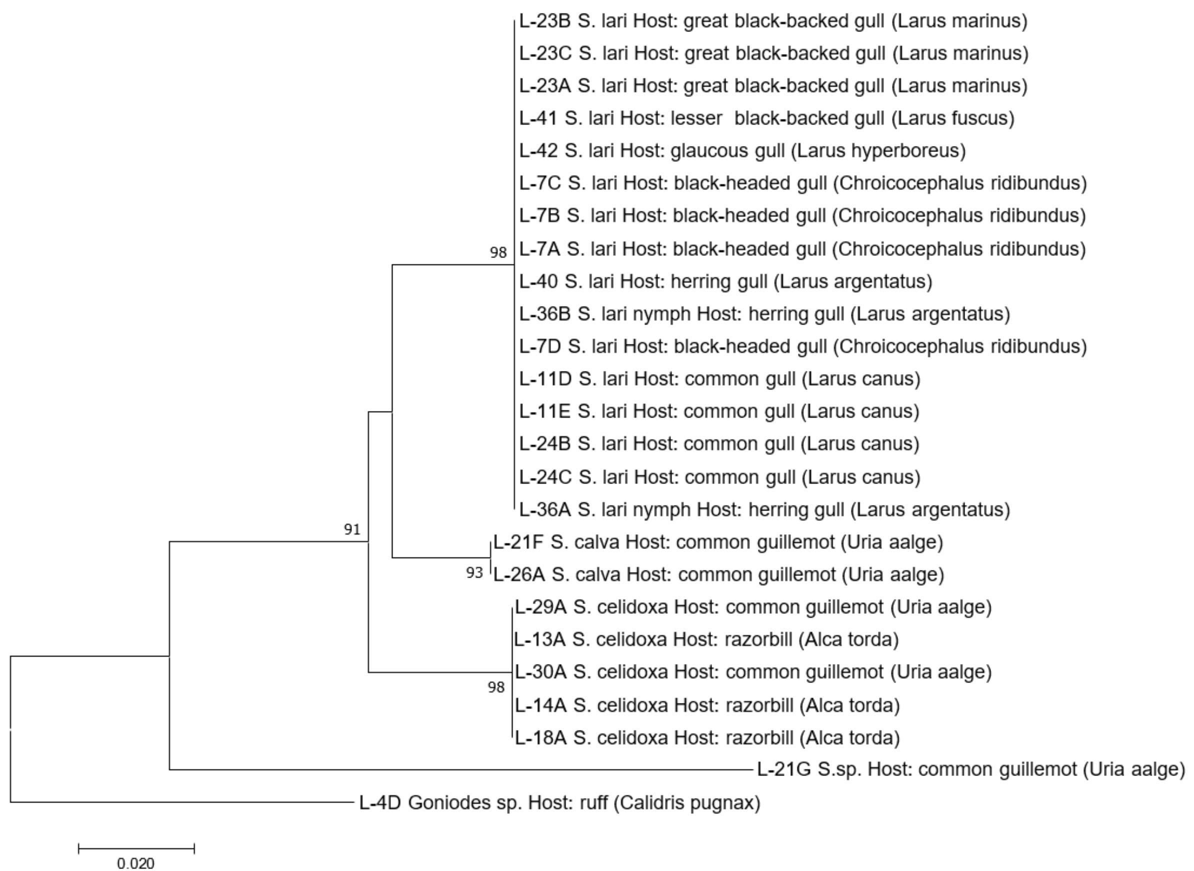

Figure 17. Neighbor-Joining tree for *Saemundssonia* species and their hosts based on sequences from the nuclear marker EF-1α. Numbers and letters before louse-host species name indicate code for this specific interaction. Numbers at nodes indicate bootstrap support. Bootstrap values below 90 were excluded. Phylogenetic outgroup is represented by *Goniodes sp.* (L-4D).

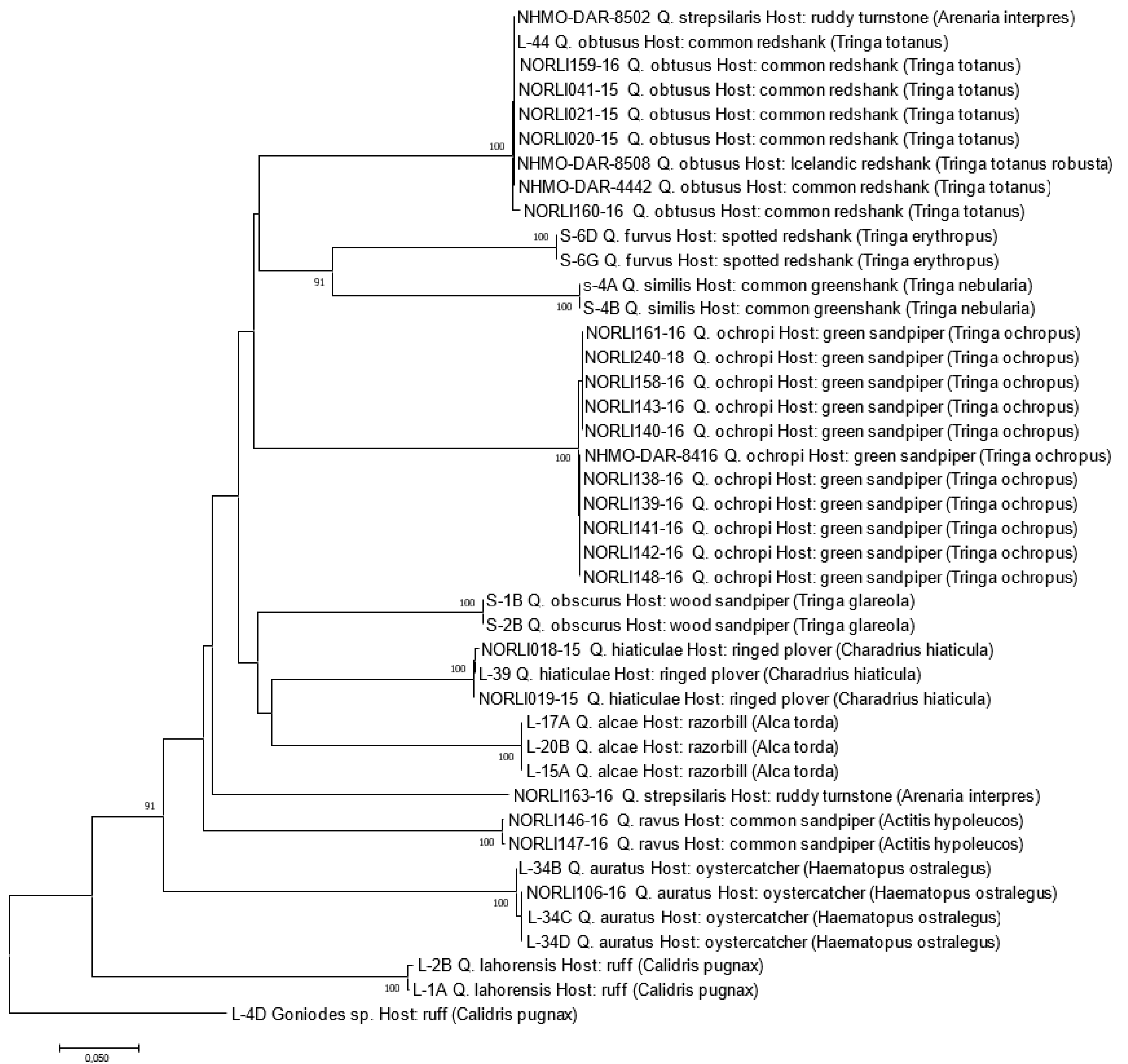

Figure 18. Neighbor-Joining tree for *Quadraceps* species based on sequences from the mitochondrial marker COI. Numbers and letters before louse-host species name indicate code for this specific interaction. Numbers at nodes indicate bootstrap support. Bootstrap values below 90 were excluded. Phylogenetic outgroup is represented by *Goniodes sp.* (L-4D).

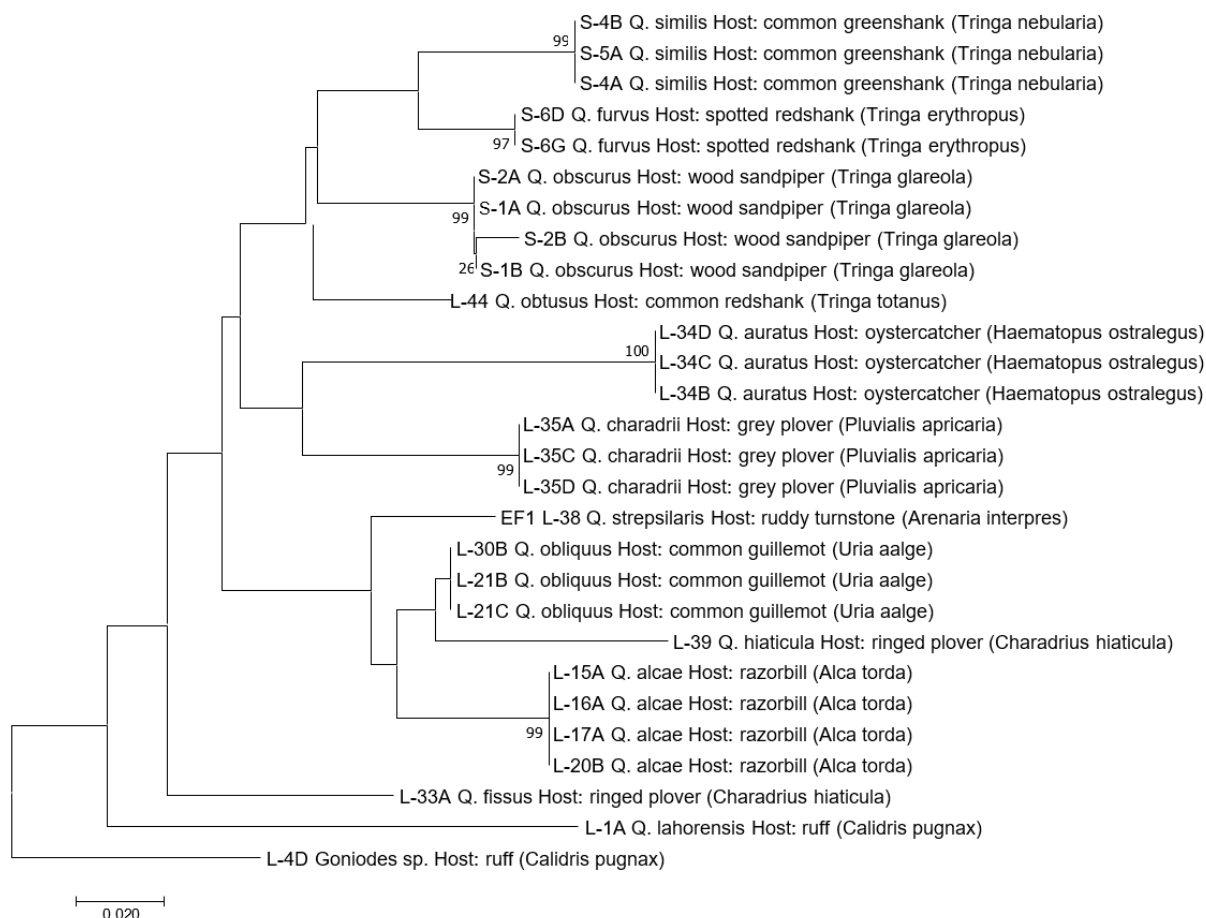

Figure 19. Neighbor-Joining tree for *Quadraceps* species based on sequences from the nuclear marker EF-1α. Numbers and letters before louse-host species name indicate code for this specific interaction. Numbers at nodes indicate bootstrap support. Bootstrap values below 90 were excluded. Phylogenetic outgroup is represented by *Goniodes sp.* (L-4D).

The collected lice mainly occured on known hosts, supported by published louse-host interactions (Table I in the Appendix), with a few exceptions. Two specimens, L-30A and L-29A, were assumed to be *S. calva* based on known louse-host interactions but recognized as *S. celidoxa* based on morphology and by clustering with other *S. celidoxa* in the EF-1α gene tree (Figure 17). The individual L-21G sampled from common guillemot (Figure 20) was identified as belonging to *Saemundssonia*, based on morphological characteristics, but I was unable to identify to species level because it differed from other *Saemundssonia* (and *Quadraceps*) collected from both common guillemots and razorbills (Figures 21 and 22). Morphologically this specimen did not match previously recorded louse-host interactions (Table I in the Appendix), nor did it match existing DNA sequences in GenBank. This specimen was genetically different from other species, and did not cluster together with other

sequences in the EF-1α gene tree (Figures 15 and 17). In the COI gene tree (Figure 18), one sequence of assumed *Q. strepsilaris* from ruddy turnstone (NHMO-DAR-8502) clustered together with *Q. obtusus*, and not with the other sample of *Q. strepsilaris* (NORLI163-16) which branched alone. This specimen was clearly misidentified based on known host interactions and was in fact *Q. obtusus*.

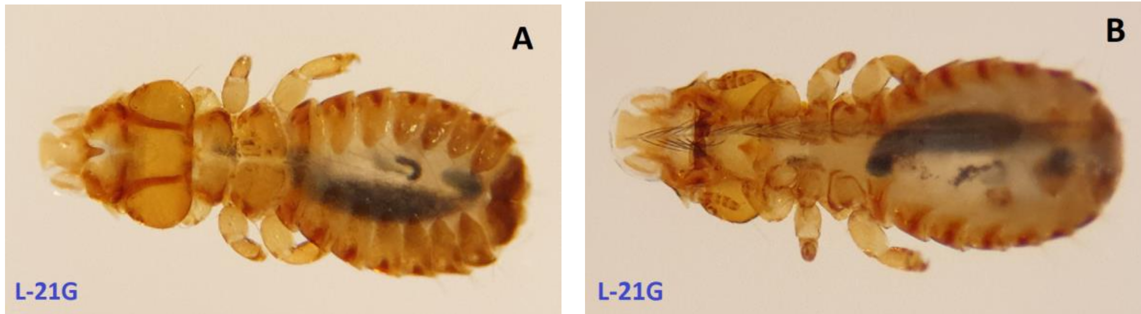

Figure 20. *Saemundssonia sp.* sampled from common guillemot (*Uria aalge*), labeled as "L-21G". **A)** dorsal view, **B)** ventral view. Photos: Lena Kristiansen.

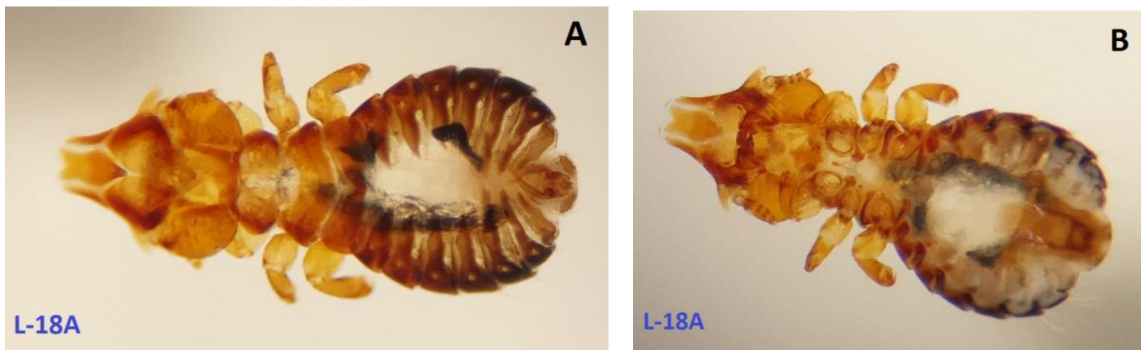

Figure 21. *Saemundssonia celidoxa* (Burmeister, 1838) sampled from razorbill (*Alca torda*), labeled as "L-18A". **A)** dorsal view, **B)** ventral view. Photos: Lena Kristiansen.

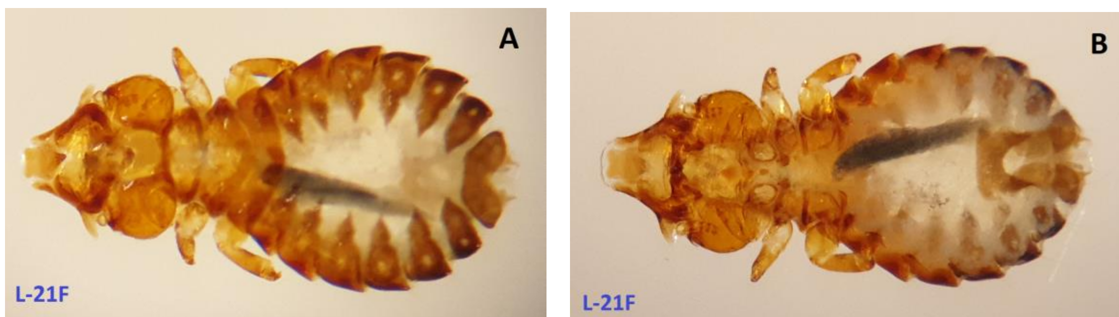

Figure 22. *Saemundssonia calva* (Kellogg, 1896a) sampled from common guillemot (*Uria aalge*), labeled as "L-21F". **A)** dorsal view, **B)** ventral view. Photos: Lena Kristiansen.

In the plumage of a ruff, I found one specimen of the ecomorphic body-louse *Goniodes sp.* (Nitzsch, 1818) (L-4D), belonging to the Ischnoceran family Goniodidae (Figure 23).

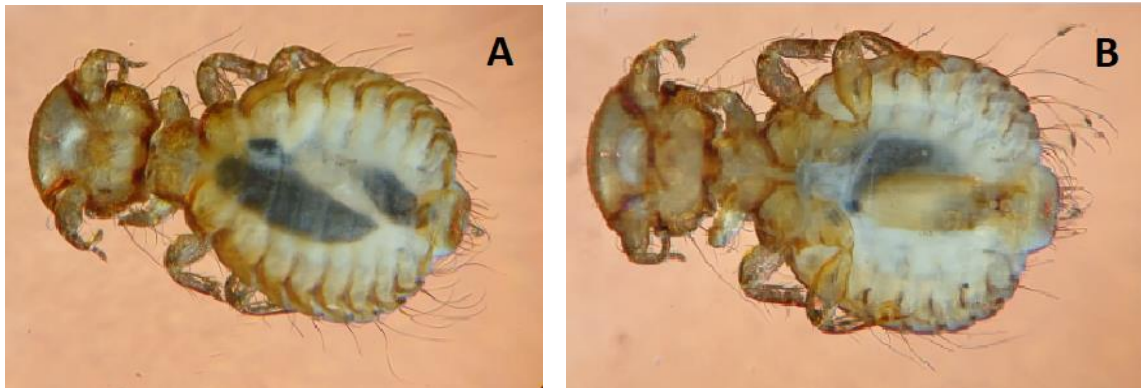

Figure 23. *Goniodes sp*. (Nitzsch, 1818) found on a dead ruff (*Calidris pugnax*). **A)** dorsal view, **B)** ventral view. Photos: Lena Kristiansen.

During this project I found 16 new species records for Norway, 14 species of *Quadraceps* and two species of *Saemundssonia*. These records include the following species: *Q. strepsilaris, Q. furvus, Q. obscurus, Q. similis, Q. ochropi, Q. Obtusus, Q. ravus, Q. hiaticula, Q. fissus, Q. auratus, Q. charadrii, Q. alcae, Q. obliquus, Q. lahorensis. S. celidoxa* and one unidentified species of *Saemundssonia* (*S. sp.*).

3.2 Host specificity

S. lari was collected from six different gull species, in the two genera *Chroicocephalus* and *Larus*: black-headed gull, herring gull, common gull, lesser black-backed gull, glaucous gull, and greater black-backed gull. *S. celidoxa* was associated with two different auk species: razorbill and common guillemot. Additionally, *S. calva* and *S. sp.* were associated with common guillemot. One *Saemundssonia* species was found on the waders: *S. tringae* collected from the red knot (Tables 5 and 6). Both gulls and auks breed colonially and attend mixed species flocks (Paragraph 1.2, Table 1). The ecomorphic head-louse *S. lari* interacted with multiple species and *S. celidoxa* interacted with two auk species. *Saemundssonia* which occurred on gregarious gulls and auks, thus seem not to be host specific.

All *Quadraceps* species were collected from both gregarious auks and more solitary waders, each associated with a different host species (Tables 5 and 6). In summary, the ecomorphic

generalist-louse *Quadraceps* was highly host specific regardless of the bird`s degree of sociality.

3.3 Abundance of lice on dead and live birds

Altogether, I examined 57 dead bird individuals belonging to eleven different Charadriiformes species represented by the genera *Chroicocephalus*, *Larus, Rissa, Alca* and *Uria* in the Lari suborder, *Calidris, Limicola, Philomachus, Arenaria*, *Tringa, Actitis, Gallinago*, *Scolopax* and *Numenius* in the Scolopaci suborder, and *Charadrius*, *Vanellus*, *Haematopus* and *Pluvialis* in the Charadrii suborder. In total, more than 400 lice were collected from the dead birds. Of the eleven dead bird species examined, seven were infested with at least one louse species from one of the two genera *Saemundssonia* and *Quadraceps* (Table 5). I observed that infestation rate varied between bird individuals, but most individuals had few lice in their plumage. I did not pick all the lice from each individual, especially when infestations rate was high. The total number of lice picked from one individual bird, varied between 1 and 50.

In addition to lice from dead birds, I included lice from 15 different living bird species collected by bird ringers (Table 6). I had no information about the number of lice collected from living birds, because these specimens were included with the purpose of increasing the number of species in the study.

Table 5. Summary of inspected dead birds from the avian order Charadriiformes and identity of collected chewing lice species from the two genera *Saemundssonia* and *Quadraceps*. * = species unidentified.

Aves Suborder	Host species (Charadriiformes)	Number of Examined Birds	Number of Infested Birds	*Saemundssonia sp.* Collected	*Quadraceps sp.* Collected
Lari	*Chroicocephalus ridibundus* (black-headed gull)	2	1	*S. lari*	---
	Larus argentatus (herring gull)	1	1	*S. lari (nymphs included)*	---
	Larus marinus (greater black-backed gull)	1	1	*S. lari*	---
	Larus canus (common gull)	8	2	*S. lari*	---
	Alca torda (razorbill)	20	11	*S. celidoxa*	*Q. alcae*
	Uria aalge (common guillemot)	8	8	*S. calva* *S. celidoxa* *S. sp.* *	*Q. obliquus*
Scolopaci	*Calidris pugnax* (ruff)	5	5	---	*Q. lahorensis*

Table 6. Chewing lice species from the two genera *Saemundssonia* and *Quadraceps* collected from Charadriiformes. All lice are sampled by bird ringers.

Aves Suborder	Host species (Charadriiformes)	Number of birds	*Saemundssonia sp.* Collected	*Quadraceps sp.* Collected
Lari	*Larus argentatus* (herring gull)	1	*S. lari*	---
	Larus fuscus (lesser black-backed gull)	1	*S. lari*	---
	Larus hyperboreus (glaucous gull)	1	*S. lari*	---
	Larus canus (common gull)	1	*S. lari*	---
Scolopaci	*Calidris canutus* (red knot)	1	*S. tringae*	---
	Arenaria interpres (ruddy turnstone)	1	---	*Q. strepsilaris*
	Tringa erythropus (spotted redshank)	1	---	*Q. furvus*
	Tringa glareola (wood sandpiper)	2	---	*Q. obscurus*
	Tringa nebularia (common greenshank)	1	---	*Q. similis*
	Tringa ochropus (green sandpiper)	1	---	*Q. ochropi*
	Tringa totanus (common redshank)	1	---	*Q. obtusus*
	Actitis hypoleucos (common sandpiper)	1	---	*Q. ravus*
Charadrii	*Charadrius hiaticula* (common ringed plover)	1 1	--- ---	*Q. hiaticula* *Q. fissus*
	Haematopus ostralegus (palearctic oystercatcher)	1	---	*Q. auratus*
	Pluvialis apricaria (European golden plover)	1	---	*Q. charadrii*

4 Discussion

In this study I found six species of *Saemundssonia* and 14 species of *Quadraceps*, all species genetically distinct from each other. All the ecomorphic generalist-lice species from the genus *Quadraceps* were found on one bird species each, while the *Saemundssonia* species collected had a broader host range. Additionally, most collected louse-host interactions were supported by known recorded interactions (Table I in the Appendix), but two new louse-host interactions were discovered largely based on molecular information: *S. celidoxa* and an unknown *Saemundssonia* species, both found on common guillemot.

<u>Identification of lice</u>

My findings suggested that *Saemundssonia* was monophyletic, whereas *Quadraceps* was divided in groups with low bootstrap support. More data are needed to be able to give any conclusions about the phylogeny of *Saemundssonia* and *Quadraceps*, and this was not the focus in this thesis. Even so, this finding supports previous studies on the systematics of these genera indicating a complex of species from several genera with unknown phylogenetic relationships, referred to as the *Quadraceps*-complex (Gustafsson *et al.* 2018). In general, molecular data obtained from COI and/or EF-1α supported the identification of *Saemundssonia* and *Quadraceps* species based on morphology and/or host interaction. *S. lari* was collected from six gull species and showed little genetic variation between the different host species both in the COI and EF-1α gene trees (Figures 16 and 17). This finding supports the traditional species delimitation based on morphology. *S. celidoxa* was collected from two auk species and also in this case no genetic variation in EF-1α between the lice collected from different hosts were observed.

Two specimens from two different individuals of common guillemot (L-29A and L-30A), were originally identified as *S. calva* based on morphology and known louse-host interactions. When using molecular methods, the two specimens grouped together with *S. celidoxa* in the EF-1α gene tree with bootstrap value of 98 (Figure 17). Additionally, compared to other specimens of *S. celidoxa* , they turned out to be morphologically similar. In this case, molecular analyses were essential for obtaining the correct species identification. The risk of contamination must be considered since common guillemots sometimes were put in common bags together with razorbills. Contamination is, however, unlikely because in this specific case, only one razorbill was present in the same bag as 12 common guillemots. Even though

many lice were still alive when bird carcasses were examined, collected lice were mainly burrowed down in the plumage. Furthermore, Ischnocera lice are known to be less mobile than Amblycera and seldom leave their dead host (Mey 2002; Clayton *et al.* 2016). I thus conclude that the finding of *S. celidoxa* on common guillemots is unlikely to be due to contamination. More closely related bird species of the same size may be more similar as a niche. Razorbills and common guillemots are both host for the same Amblyceran louse species *Austromenopon nigropleurum* (Price *et al.* 2003b) which might suggest that the two bird species have common niche properties that might facilitate survival of other common louse species.

Molecular data were also an important tool in the identification of another louse individual collected from common guillemot. This specimen was identified to genus, but not to species, because I could not find any species match based on morphological characteristics and host interactions. However, based on molecular analyses, the sequence differed greatly from other species in the EF-1α gene tree (Figure 17), which supported the idea that this was a separate species. Finally, a louse collected from ruddy turnstone (NHMO-DAR-8502) was another example where host association was not the best method to classify louse to species. This specimen was identified as *Q. strepsilaris* based on genus morphology and known host interactions, but in the molecular analyses it clustered together with *Q. obtusus*. Both *Q. strepsilaris* and *Q. obtusus* were found on ruddy turnstone, but as far as I know the association between *Q. obtusus* and ruddy turnstone is not recorded in available publications (Price *et al.* 2003b).

Traditionally, the birds have been more studied than their parasites, and lice might have been overlooked in previous studies, or misidentified solely based on known host-louse interactions. This study has shown that species identification should not be based solely on louse-host interactions but complimented by molecular methods such as DNA barcoding in order to distinguish species correctly. The most ideal would be to obtain sequences from both mitochondrial and nuclear genes, because they have different rates of sequence evolution, and mitochondrial genes record only maternal heredity history.

Ecomorphs and host specificity

All 14 *Quadraceps* species infesting waders and auks were found on different host species each, suggesting that *Quadraceps* is highly host specific. *S. lari* was found on six different

gull species and *S. celidoxa* was found on two species of auks, suggesting that *Saemundssonia* is less host specific. At bird order level, the same pattern of host specificity between the two genera of lice are described in Price *et al.* (2003b): *Saemundssonia* parasitize five orders of birds, and *Quadraceps* parasitize two. Among gulls, a study performed by Yamagishi *et al.* (2014), also suggested that *Saemundssonia* was less host specific and *Quadraceps* highly host specific. Contradictory to my findings, Price *et al.* (2003a) found that *Saemundssonia* among auks was highly host specific, at least at the bird-genus level. Nine out of ten species of *Saemundssonia* associated with only one genus of alcids each, for instance *S. calva* associated with two species of *Uria*, and *S. grylle* associated with two species of *Cepphus* (Price *et al.* 2003a). Even though *S. lari* and *S. celidoxa* have a broader host range, it is possible that host specificity among *Saemundssonia* vary between bird genera on which they parasitize. Even though more data is needed, it seems as if *Saemundssonia* possesses qualities which to a greater extent makes them able to exhibit a broader range of hosts than *Quadraceps*. One important difference between *Saemundssonia* and *Quadraceps* is the use of different microhabitats on the bird. Characteristics of feathers are important environmental components for chewing louse survival because feathers are substrates for feeding, ovipositing, locomotion and hiding from preening. Compared to *Quadraceps*, which lives at different places on the body of a bird, *Saemundssonia* can more easily escape preening and reproduce on novel hosts because they live on the head or neck of the bird. The difference in use of microhabitat might explain why *Saemundssonia* generally are less host specific than *Quadraceps* (Yamagishi *et al.* 2014).

Both gulls and auks breed colonially and roost in mixed species flocks, facilitating direct horizontal transmission of lice. Avian cycles of migration and breeding follow an annual rhythm (Gwinner 1996). Lice have shown synchronized reproduction and activity with that of their hosts by detecting changes in host sociability during periods when hosts are gregarious, increasing reproduction and chances of dispersal through horizontal and/or vertical transmission (Clayton & Tompkins 1994). Due to increased body-to-body contact among colonial birds, the rate of horizontal transmission of lice between unrelated host individuals is higher among social birds, even though this pattern is less pronounced among Ischnocera (Rózsa *et al.* 1996). Horizontal transmission is also higher among social individuals within single species of birds (Whiteman & Parker 2004). Even though flocking behaviour increases the chance of horizontal transmission, studies about the relationship between bird sociality and parasite abundance give contrasting results. Some studies find that birds living in flocks

are highly parasitized (Brown & Brown 1986; Côtè & Poulin 1995), while others do not find such correlation (Rózsa *et al.* 1996; D`Amico & Barbosa 2011). Even though different species of Scolopaci and Charadrii meet at common sites during migration or common roots, transfer of lice might be hindered between hosts of different size (Tompkins & Clayton 1999; Johnson *et al.* 2005; Bush & Clayton 2006). Some studies suggest that wing lice generally cannot establish on larger or smaller novel hosts (Tompkins & Clayton 1999; Bush & Clayton 2006). Whether this is transferable for generalist lice is unknown (Gustafsson & Olsson 2017) even though Yamagishi *et al.* (2014) found that the body size of *Quadraceps* corresponded to the body size of their hosts. Whether the degree of host specificity among *S. lari* and *S. celidoxa* can be influenced by the bird's degree of sociality is uncertain because they were both found on typically gregarious birds. *Quadraceps* associated with both gregarious auks and solitary waders (Paragraph 1.2, Table 1), which might indicate that *Quadraceps* was highly host specific regardless of the bird's degree of sociality.

Three different *Saemundssonia* species were collected from the common guillemot: *S. calva*, *S. celidoxa* and *S. sp.*, two different *Quadraceps* species were collected from common ringed plover: *Q. hiaticula* and *Q. fissus,* and two different *Quadraceps* species were collected from ruddy turnstone: *Q. strepsilaris* and *Q. obtusus*. All louse species were found on different host individuals, except *S. calva* and *S. sp* which were sampled from the same bird (L-21). Gause`s law states that two species with equal resource requirements cannot occupy the same niche (Gause 1934). By this principle, competition will drive one species to extinction, or the species will evolve adaptations which allow them to utilize non-overlapping microhabitat. Finding more than one species of lice from the same genus collected from the same host species is not unusual. For example, the two louse species *Columbicola baculoides* and *Columbicola macrourae* have been found together on mourning dove, *Zenaida macroura*, (Columbiformes) (Galloway & Palma 2008). Even two pairs of species of lice within the same genus have been found on sora, *Porzana Carolina* (Gruiformes): *Fulicoffula americana* and *Fulicoffula distincta*, and *Rallicola mystax* and *Rallicola subporzanae* (Galloway 2004). Freedom from competition with resident parasites increases the chance of successful establishment on novel hosts (Hudault *et al.* 2001; Dillon *et al.* 2005). The ability for two species of the same genus or ecomorph, to exist together on one host seems to contradict Gause`s law.

In summary, the degree of host specificity differed between ecomorphic head- and generalist lice. Even though flocking behaviour among birds increases the chances of direct horizontal transmission of lice, it was difficult to make any firm conclusions about whether the degree of host specificity of lice differed between gregarious and solitary birds.

Goniodes sp.

Goniodes species do not use birds from Charadriiformes as their regular natural host but are restricted to hosts in the orders Galliformes (Johnson *et al.* 2001; Price *et al.* 2003) and Columbiformes (Gustafsson *et al.* 2018). In Norway, *Goniodes* species have been recorded from rock ptarmigan (*Lagopus muta*), willow ptarmigan (*Lagopus lagopus*) and capercaillie (*Tetrao urogallus*) (Mehl 1981; Mehl *et al.* 1982). The finding of a specimen of *Goniodes sp.* on a ruff was therefore unexpected. The specimen was identified as *Goniodes sp.* by blast search in GenBank, and differed substantially from both *Saemundssonia* and *Quadraceps,* in the COI and EF-1α genes, and was therefore selected as a phylogenetic outgroup.

The inspected dead ruff was collected from Gardermoen airport and stored in a separate bag. The procedure to avoid contamination (paragraph 2.1.2) was followed and I thus conclude that the finding of *Goniodes sp.* on ruff is unlikely to be due to contamination. When alive, the ruff most likely does not physically meet species from Galliformes or Columbiformes due to different habitat preferences. Louse flies have been recorded from ruff (Bartos *et al.* 2020), and phoresy might thus be an explanation for this louse-host association. Whether this louse species can survive and reproduce on ruff is uncertain because the louse must escape from the birds preening and compete with other louse species. More data are needed to make any conclusions about whether this is a new, well-established louse-host interaction.

5 Conclusion

Species determination of chewing lice has to a large extent been based on already known louse-host interactions and morphology. In this study DNA sequence data were used in addition to the traditional methods to investigate the species of *Saemundssonia* and *Quadraceps* hosted by birds in the order Charadriiformes. The results show that overall the DNA sequences of COI and EF-1α support the species identification of lice based on host and morphology. The two different markers also show the same pattern, indicating that DNA barcoding are important tools that makes it easier to distinguish species and detect uncommon louse-host interactions. Today, very few DNA sequences of chewing lice are available to compare species, and more work is needed to build a reference library of DNA barcode sequences from *Saemundssonia, Quadraceps* and other chewing lice genera, to effectively use it for identification of species.

The *Quadraceps* species collected from waders (Scolopaci and Charadrii) and auks (*Uria aalge* and *Alca torda*) in this study seemed to be highly host specific, parasitizing one host species each. In comparison, *Saemundssonia* collected from gulls and auks (Lari) parasitized a broader range of host species and seemed to be less host specific. *Saemundssonia* is an ecomorphic head-louse and escape the birds' preening by living on the head or neck of their host, perhaps making survival on a novel host more likely. *Quadraceps* is an ecomorphic generalist-louse which to a greater extent depend on morphological and behavioral features to escape preening on novel hosts. The difference in host specificity can also be explained by the host behavior, because

for persistence of a chewing louse lineage, transmission to a new host is essential. Gulls and auks breed colonially and roost in mixed species flocks facilitating inter- and intraspecific direct horizontal transmission. Even though *Saemundssonia* have a broader host range than *Quadraceps*, it is unclear whether this is affected by the degree of sociality of the bird, the difference in microhabitat use, or both. To be able to conclude on which of these factors that determine the difference in host specificity in the two genera, more work is needed to achieve a better understanding of relationships between *Saemundssonia* and *Quadraceps* lice and their hosts sosiality.

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Appendix

Table I. List of bird species sampled in this project and species from the genera *Saemundssonia* and *Quadraceps* known to occur on the bird species. Broad billed sandpiper (*Limicola falcinellis*) does not host lice from either *Saemundssonia* or *Quadraceps*. The right column shows total number of lice species for each bird species. The data in this table is obtained from Smith et al (2021). * = sample with no DNA-data.

Bird Suborder	Bird Species	*Quadraceps* Species	*Saemundssonia* Species	Totally number of Host – Louse interactions
Lari	Chroicocephalus ridibundus (black-headed gull)	Q. punctatus punctatus (Burmeister, 1838)	S. (S.) lari (Fabricius [O.], 1780)	5
	Larus argentatus (European herring gull)	Q. ornatus striolatus (Nitzsch [In Giebel], 1866)	S. (S.) lari (Fabricius [O.], 1780)	4
	Larus marinus (great black-backed gull)	Q. ornatus striolatus (Nitzsch [In Giebel], 1866)	S. (S.) lari (Fabricius [O.], 1780)	4
	Larus fuscus (lesser black-backed gull)	Q. punctatus regressus (Timmermann, 1952a)	S. (S.) lari (Fabricius [O.], 1780)	4
	Larus hyperboreus (glaucous gull)	Q. ornatus striolatus (Nitzsch [In Giebel], 1866)	S. (S.) lari (Fabricius [O.], 1780)	4
	Larus canus (common gull)	Q. punctatus regressus (Timmermann, 1952a) Q. ornatus ornatus (Grube, 1851)	S. (S.) lari (Fabricius [O.], 1780)	5
	Rissa tridactyla (black-legged kittiwake) *	Q. ornatus paulschulzei (Timmermann, 1949) Q. ornatus lineolatus (Nitzsch [In Giebel], 1866)	S. (S.) lari (Fabricius [O.], 1780)	5
	Alca torda (razorbill)	Q. alcae (Denny, 1842)	S. (S.) celidoxa (Burmeister, 1838)	3
	Uria aalge (common guillemot)	Q. obliquus (Mjöberg, 1910b)	S. (S.) calva (Kellogg, 1896a)	3
Scolopaci	Actitis hypoleucos (common sandpiper)	Q. ravus (Kellogg, 1899)	S. (S.) platygaster frater (Giebel, 1874)	4
	Arenaria interpres (ruddy turnstone)	Q. strepsilaris (Denny, 1842)	S. (S.) tringae (Fabricius [O.], 1780) S. (S.) platygaster stenrami (Timmermann, 1969b)	6
	Calidris alba (sanderling) *	missing	S. (S.) tringae (Fabricius [O.], 1780) S. (S.) platygaster jadwigae (Timmermann, 1969b)	5
	Calidris alpina (dunlin) *	missing	S. (S.) tringae (Fabricius [O.], 1780)	5
	Calidris canutus (red knot)	missing	S. (S.) tringae (Fabricius [O.], 1780) S. (S.) platygaster islandica (Timmermann, 1951a)	7
	Calidris ferruginea (curlew sandpiper) *	missing	S. (S.) tringae (Fabricius [O.], 1780)	5
	Calidris maritima (purple sandpiper) *	missing	S. (S.) tringae (Fabricius [O.], 1780)	5
	Calidris minuta (little stint) *	missing	S. (S.) platygaster nitzschi (Giebel, 1866) S. (S.) tringae (Fabricius [O.], 1780)	5
	Calidris pugnax (ruff)	Q. lahorensis (Ansari, 1955b)	S. (S.) tringae (Fabricius [O.], 1786) S. (S.) platygaster ashi (Timmermann [G.], 1955a)	7
	Limicola falcinellus (broad-billed sandpiper)	missing	missing	1
	Limosa lapponica (bar-tailed godwit) *	missing	S. (S.) limosae (Denny, 1842)	6
	Numenius arquata (western/Eurasian curlew)	missing	S. (S.) scolopacis phaeopodis humeralis (Denny, 1842)	5

	Scolopax rusticola (Eurasian woodcock) *	missing	S. (S.) clayae (Hopkins, 1949)	5
	Tringa erythropus (spotted redshank)	Q. furvus (Burmeister, 1838)	S. (S.) platygaster nitzschi (Giebel, 1866)	4
	Tringa glareola (wood sandpiper)	Q. obscurus (Burmeister, 1838)	S. (S.) platygaster cordiceps (Giebel, 1874)	3
	Tringa nebularia (common greenshank)	Q. similis (Giebel, 1866)	S. (S.) platygaster nitzschi (Giebel, 1866)	3
	Tringa ochropus (green sandpiper)	Q. ochropi (Denny, 1842)	S. (S.) platygaster nitzschi (Giebel, 1866)	4
	Tringa totanus (common redshank)	Q. obtusus (Kellogg & Kuwane, 1902)	S. (S.) platygaster mollis (Nitzsch [In Giebel], 1874)	4
Charadrii	Charadrius hiaticula (ringed plover)	Q. hiaticulae (Fabricius [O], 1780) Q. fissus (Burmeister, 1838)	S. (S.) platygaster platygaster (Denny, 1842)	5
	Haematopus ostralegus (Palearctic oystercatcher)	Q. auratus (De Haan, 1829)	S. (S.) haematopi (Giebel, 1874)	5
	Pluvialis apricaria (European golden plover)	Q. charadrii (Linnaeus, 1758)	S. (S.) conica conica (Denny, 1842)	3

Table II: List of species from the two genera *Saemundssonia* and *Quadraceps* and their host species, together with information on obtained cytochrome oxidase subunit I (COI) and elongation factor 1α (EF-1α) markers. Sample codes labeled as "NORLI" are lice collected during the project "Featherlice in Norway". Sample codes labeled as "L" are lice collected by me and" codes labeled as S" by bird ringers during this project. One sample represented as a phylogenetic outgroup, *Goniodes sp.* (L-4D), is included in table.

Louse Species	Aves Species	Sample Code Louse	NHMO-DAR (Louse)	NHMO-BI (Aves)	Ring Number (Aves)	CO1	EF-1α	Date Collection Louse	Date Collection Aves
Goniodes sp.	*Calidris pugnax*	L-4D	16398	84984	---	X	X	16.01.2020	21.07.2014
Quadraceps alcae	*Alca torda*	L-15A	16390	14259	---	X	X	08.11.2020	26.09.2007
Quadraceps alcae	*Alca torda*	L-16A	16396	14289	---	---	X	08.11.2020	26.09.2007
Quadraceps alcae	*Alca torda*	L-17A	16420	14274	---	X	X	13.10.2020	26.09.2007
Quadraceps alcae	*Alca torda*	L-20B	16424	14267	---	X	X	13.10.2020	26.09.2007
Quadraceps auratus	*Haematopus ostralegus*	L-34B	16454	---	586149	X	X	00.11.2020	10.09.2020
Quadraceps auratus	*Haematopus ostralegus*	L-34C	16455	---	586149	X	X	00.11.2020	10.09.2020
Quadraceps auratus	*Haematopus ostralegus*	L-34D	16456	---	586149	X	X	00.11.2020	10.09.2020
Quadraceps auratus	*Haematopus ostralegus*	NORLI106-16	8356	---	5191997	X	---	25.05.2015	25.05.2015
Quadraceps charadrii	*Pluvialis apricaria*	L-35A	16457	---	8B97350/J74	---	X	08.09.2020	08.09.2020
Quadraceps charadrii	*Pluvialis apricaria*	L-35C	16459	---	8B97350/J74	---	X	08.09.2020	08.09.2020
Quadraceps charadrii	*Pluvialis apricaria*	L-35D	16460	---	8B97350/J74	---	X	08.09.2020	08.09.2020
Quadraceps fissus	*Charadrius hiaticula*	L-33A	16452	---	---	---	X	08.09.2020	08.09.2020
Quadraceps furvus	*Tringa erythropus*	S-6D	16416	---	7218870	X	X	23.08.2017	23.08.2017
Quadraceps furvus	*Tringa erythropus*	S-6G	16419	---	7218870	X	X	23.08.2017	23.08.2017
Quadraceps hiaticulae	*Charadrius hiaticula*	L-39	4437	---	8B46616	---	X	25.08.2013	25.08.2013
Quadraceps hiaticulae	*Charadrius hiaticula*	NORLI018-15	4435	---	8B46616	X	---	25.08.2013	25.08.2013
Quadraceps hiaticulae	*Charadrius hiaticula*	NORLI019-15	4436	---	8B46616	X	---	25.08.2013	25.08.2013
Quadraceps lahorensis	*Calidris pugnax*	L-1A	16371	84982	---	X	X	16.01.2020	21.07.2014
Quadraceps lahorensis	*Calidris pugnax*	L-2B	16397	84980	---	X	---	16.01.2020	21.07.2014
Quadraceps obliquus	*Uria aalge*	L-21B	16426	14278	---	---	X	13.10.2020	26.09.2007
Quadraceps obliquus	*Uria aalge*	L-21C	16427	14278	---	---	X	13.10.2020	26.09.2007
Quadraceps obliquus	*Uria aalge*	L-30B	16451	105375	---	---	X	10.11.2020	03.11.2020
Quadraceps obscurus	*Tringa glareola*	S-1A	16378	---	8B81951	---	X	16.08.2017	16.08.2017
Quadraceps obscurus	*Tringa glareola*	S-1B	16411	---	8B8195	X	X	16.08.2017	16.08.2017
Quadraceps obscurus	*Tringa glareola*	S-2A	16379	---	8B81957	---	X	23.08.2017	23.08.2017
Quadraceps obscurus	*Tringa glareola*	S-2B	16412	---	8B81957	X	X	23.08.2017	23.08.2017
Quadraceps obtusus	*Tringa totanus*	L-44	4443	---	7544305	X	X	25.08.2013	25.08.2013
Quadraceps obtusus	*Tringa totanus*	NORLI020-15	4441	---	7544305	X	---	25.08.2013	25.08.2013
Quadraceps obtusus	*Tringa totanus*	NORLI021-15	4442	---	7544305	X	---	25.08.2013	25.08.2013
Quadraceps obtusus	*Tringa totanus*	NORLI041-15	4518	---	7544304	X	---	25.08.2013	25.08.2013
Quadraceps obtusus	*Tringa totanus*	NORLI159-16	8468	---	7608355	X	---	07.08.2015	07.08.2015
Quadraceps obtusus	*Tringa totanus*	NORLI160-16	8469	---	7608355	X	---	07.08.2015	07.08.2015
Quadraceps obtusus	*Tringa totanus robusta*	NHMO-DAR-8508	8508	---	DA45441	X	---	28.08.2015	28.08.2015
Quadraceps obtusus	*Tringa totanus*	NHMO-DAR-4442	4442	---	7544305	X	---	25.08.2013	25.08.2015

Quadraceps ochropi	*Tringa ochropus*	NORLI138-16	8415	---	8B46858	X	---	01.08.2015	03.08.2015
Quadraceps ochropi	*Tringa ochropus*	NORLI139-16	8416	---	8B46858	X	---	01.08.2015	03.08.2015
Quadraceps ochropi	*Tringa ochropus*	NORLI140-16	8419	---	8B46859	X	---	03.08.2015	03.08.2015
Quadraceps ochropi	*Tringa ochropus*	NORLI141-16	8420	---	8B46859	X	---	03.08.2015	03.08.2015
Quadraceps ochropi	*Tringa ochropus*	NORLI142-16	8421	---	8B46859	X	---	03.08.2015	03.08.2015
Quadraceps ochropi	*Tringa ochropus*	NORLI143-16	8422	---	8B46859	X	---	03.08.2015	03.08.2015
Quadraceps ochropi	*Tringa ochropus*	NORLI148-16	8440	---	8B46859	X	---	03.08.2015	03.08.2015
Quadraceps ochropi	*Tringa ochropus*	NORLI158-16	8466	---	8B46860	X	---	03.08.2015	03.08.2015
Quadraceps ochropi	*Tringa ochropus*	NORLI161-16	8475	---	8B46860	X	---	03.08.2015	03.08.2015
Quadraceps ochropi	*Tringa ochropus*	NORLI240-18	292388	---	TriOch160722	X		22.07.2016	22.07.2016
Quadraceps ochropi	*Tringa ochropus*	NHMO-DAR-8416	8416	---	8B46858	X	---	01.08.2015	01.08.2015
Quadraceps ravus	*Actitis hypoleucos*	NORLI146-16	8437	---	8B81506	X	---	03.08.2015	03.08.2015
Quadraceps ravus	*Actitis hypoleucos*	NORLI147-16	8438	---	8B81506	X	---	03.08.2015	03.08.2015
Quadraceps similis	*Tringa nebularia*	S-4A	16381	---	7218365	X	X	26.07.2017	26.07.2017
Quadraceps similis	*Tringa nebularia*	S-4B	16415	---	7218365	X	X	26.07.2017	26.07.2017
Quadraceps similis	*Tringa nebularia*	S-5A	16382	---	7218369	---	X	23.08.2017	23.08.2017
Quadraceps strepsilaris	*Arenaria interpres*	L-38	8498	---	7608439	---	X	28.08.2015	28.08.2015
Quadraceps strepsilaris	*Arenaria interpres*	NORLI163-16	8480	---	7608364	X	---	12.08.2015	12.08.2015
Quadraceps strepsilaris	*Arenaria interpres*	NHMO-DAR-8502	8502	---	7608362	X	---	12.08.2015	12.08.2015
Saemundssonia calva	*Uria aalge*	L-21F	16430	14278	---	X	X	13.10.2020	26.09.2007
Saemundssonia calva	*Uria aalge*	L-21G	16431	14278	---	---	X	13.10.2020	26.09.2007
Saemundssonia calva	*Uria aalge*	L-26A	16444	105396	---	X	X	10.11.2020	10.11.2020
Saemundssonia celidoxa	*Alca torda*	L-13A	16387	14260	---	X	X	07.10.2020	26.09.2007
Saemundssonia celidoxa	*Alca torda*	L-14A	16389	14301	---	X	X	07.10.2020	26.09.2007
Saemundssonia celidoxa	*Alca torda*	L-18A	16421	14276	---	X	X	13.10.2020	26.09.2007
Saemundssonia celidoxa	*Uria aalge*	L-29A	16448	105370	---	---	X	10.11.2020	03.11.2020
Saemundssonia celidoxa	*Uria aalge*	L-30A	16450	105375	---	---	X	10.11.2020	03.11.2020
Saemundssonia clayae	*Scolopax rusticola*	L-6C	16373	105415	---	---	X	17.01.2020	27.12.2019
Saemundssonia clayae	*Scolopax rusticola*	L-6D	16400	105415	---	---	X	17.01.2020	27.12.2019
Saemundssonia clayae	*Scolopax rusticola*	L-6E	16401	105415	---	---	X	17.01.2020	27.12.2019
Saemundssonia clayae	*Scolopax rusticola*	L-8F	16375	102707	---	---	X	17.01.2020	28.06.2010
Saemundssonia lari	*Chroicocephalus ridibundus*	L-7A	16365	105416	---	X	X	17.01.2020	12.06.2019
Saemundssonia lari	*Chroicocephalus ridibundus*	L-7B	16374	105416	---	X	X	17.01.2020	12.06.2019
Saemundssonia lari	*Chroicocephalus ridibundus*	L-7C	16403	105416	---	X	X	17.01.2020	12.06.2019
Saemundssonia lari	*Chroicocephalus ridibundus*	L-7D	16404	105416	---	X	X	17.01.2020	12.06.2019
Saemundssonia lari	*Larus argentatus*	L-36A	16462	105412	---	X	X	26.11.2020	26.11.2020
Saemundssonia lari	*Larus argentatus*	L-36B	16463	105412	---	X	X	26.11.2020	26.11.2020
Saemundssonia lari	*Larus argentatus*	L-40	8519	---	JL006	X	X	03.11.2013	03.11.2013
Saemundssonia lari	*Larus argentatus*	NORLI180-16	8516	---	JL006	X	---	03.11.2013	03.11.2013
Saemundssonia lari	*Larus argentatus*	NORLI181-16	8517	---	JL006	X	---	03.11.2013	03.11.2013

Saemundssonia lari	*Larus argentatus*	NORLI182-16	8518	---	JL006	X	---	03.11.2013	03.11.2013
Saemundssonia lari	*Larus canus*	L-11D	16409	105411	---	X	X	10.08.2020	04.11.2018
Saemundssonia lari	*Larus canus*	L-11E	16410	105411	---	X	X	10.08.2020	04.11.2018
Saemundssonia lari	*Larus canus*	L-24B	16439	---	---	X	X	21.10.2020	Unknown
Saemundssonia lari	*Larus canus*	L-24C	16440	---	---	X	X	21.10.2020	Unknown
Saemundssonia lari	*Larus canus*	NORLI183-16	8522	---	J2EY	X	---	02.09.2013	02.09.2013
Saemundssonia lari	*Larus canus*	NORLI271-18	292213	---	JC 312_018	X	---	08.09.2016	08.09.2016
Saemundssonia lari	*Larus canus*	NORLI272-18	292214	---	JC 312_019	X	---	08.09.2016	08.09.2016
Saemundssonia lari	*Larus fuscus*	L-41	8534	---	JE5K	---	X	17.06.2012	17.06.2012
Saemundssonia lari	*Larus fuscus*	NORLI188-16	8531	---	JE5K	X	---	17.06.2012	17.06.2012
Saemundssonia lari	*Larus fuscus*	NORLI189-16	8532	---	JE5K	X	---	18.06.2012	18.06.2012
Saemundssonia lari	*Larus fuscus*	NORLI190-16	8533	---	JE5K	X	---	19.06.2012	19.06.2012
Saemundssonia lari	*Larus hyperboreus*	L-42	8528	---	JP380	---	X	15.04.2012	15.04.2012
Saemundssonia lari	*Larus hyperboreus*	NORLI185-16	8525	---	JP380	X	---	15.04.2012	15.04.2012
Saemundssonia lari	*Larus hyperboreus*	NORLI186-16	8526	---	JP380	X	---	15.04.2012	15.04.2012
Saemundssonia lari	*Larus hyperboreus*	NORLI187-16	8527	---	JP380	X	---	15.04.2012	15.04.2012
Saemundssonia lari	*Larus marinus*	L-23A	16435	35342	---	X	X	21.10.2020	00.00.2008
Saemundssonia lari	*Larus marinus*	L-23B	16436	35342	---	X	X	21.10.2020	00.00.2008
Saemundssonia lari	*Larus marinus*	L-23C	16437	35342	---	X	X	21.10.2020	00.00.2008
Saemundssonia tringae	*Calidris canutus*	NORLI093-15	4723	---	7544958	X	---	29.08.2014	29.08.2014