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Deutonymphs of *Neottialges caparti* Fain (Astigmata: Hypoderatidae) from North American Northern Gannet (*Morus bassanus*)

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Abstract

Deutonymphs of the mite family Hypoderatidae are subdermal parasites of vertebrates, primarily birds. Here we report the presence of deutonymphs of *Neottialges caparti* Fain (no common name) in the subcutis of Northern Gannet (*Morus bassanus* (L.)) from Atlantic Canadian waters. We observed mites in 39% of 90 birds whose skin samples were examined microscopically. Our observations represent both the first record of *N. caparti* in Northern Gannet from the western North Atlantic and the second report of *N. caparti* since its initial description in 1967 from two Northern Gannets in Belgium.

Key words: Arachnida; Acariformes; Aves; deutonymphs; Hypoderatidae; *Neottialges caparti*; mites; new distribution record; Northern Gannet; subcutaneous parasites

Résumé

Les deutonymphes de la famille d'acariens Hypoderatidae sont des parasites sous-cutanés des vertébrés, surtout des oiseaux. Nous décrivons ici la présence de deutonymphes de *Neottialges caparti* Fain (pas de nom commun) dans le tissu sous-cutané de Fous de Bassan (*Morus bassanus* (L.)) des eaux canadiennes de l'Atlantique. Nous avons observé des acariens dans 39 % de 90 oiseaux dont des échantillons de peau furent examinés microscopiquement. Nos observations représentent la première mention de *N. caparti* chez les Fous de Bassan de l'Atlantique nord-ouest et la seconde mention de *N. caparti* depuis sa description originale en 1967 chez deux Fous de Bassan de la Belgique.

Mots-clés : Arachnida; Acariformes; Aves; deutonymphes; Fou de Bassan; Hypoderatidae; *Neottialges caparti*; nouvelle mention de distribution; parasites sous-cutanés

Introduction

Metazoan parasites are ubiquitous in free-living wildlife and, as a group, can occupy essentially all possible niches on and in a host organism. The cost to a host may vary from negligible to lethal depending on several factors, including life stage of the parasite, host species, integrity of the host's immune system at the time of exposure, and number of individual parasites to which the host is exposed at a given time (Wobeser 2008). Metazoan parasites that are small, infect hosts that are difficult to access, inhabit well-concealed niches within their host, and cause few to no apparent detrimental effects to the host can easily go unnoticed and may thus remain poorly documented.

Northern Gannet (Sulidae: *Morus bassanus* (L.)) is a piscivorous bird that feeds by plunge-diving. It is broadly distributed along the eastern and western shores of the North Atlantic Ocean (Mowbray 2020; BirdLife International 2023). It is typically a coastal species that rarely comes over land except to breed. Its North American population is estimated at approximately 230 000 adults breeding in six colonies, all located in the Gulf of St. Lawrence and along the southeastern coast of insular Newfoundland, Canada (Chardine *et al.* 2013). The adults are monogamous and philopatric, the breeding pair reusing the same nest in successive years (Mowbray 2020). Arriving at the colonies in March–April, the adults start adding material to the nest, collecting it from the nesting

island, adjacent islands, or the sea. A single egg is laid, and incubation and care of the young are shared by both parents. The nests are evenly spaced and nearly touching, with their centres 60–80 cm apart. The young fledge in mid-September or later at ~90 days of age and the population migrates to waters along the southeastern United States and the Gulf of Mexico to overwinter (Chapdelaine 1996; Mowbray 2020).

Here we report the occurrence of typically inconspicuous subcutaneous mites in a large number of Northern Gannets examined over 28 years.

Methods

As part of a health surveillance program in free-living wildlife (Leighton *et al.* 1997), fresh or frozen carcasses of Northern Gannets that had been found seriously injured, sick, or dead along the shores of the three Canadian Maritime provinces (New Brunswick, Nova Scotia, and Prince Edward Island) were submitted for post-mortem examination to the Atlantic regional centre of the Canadian Wildlife Health Cooperative, based at the Atlantic Veterinary College, University of Prince Edward Island. Between 1990 and 2017, 313 carcasses were necropsied by P.-Y.D. Depending on the state of preservation of the carcasses, samples from observed lesions and standard samples from a variety of organs and tissues, including one ~2.5-cm-long section of skin from the mid-ventral region of the abdomen, were collected. These samples were fixed in 10% neutral-buffered formalin, dehydrated in graded alcohol and xylene, and embedded in paraffin; 5-µm-thick sections were cut from the paraffin blocks and stained with hematoxylin and eosin for light microscopic examination. The relative age of the birds (hatch-year [HY], immature [1–4 years], adult [≥5 years]) was determined based on plumage (Harrison 1983; Sibley 2000), presence or absence of a bursa of Fabricius, and degree of development of reproductive organs. The nutritional condition was subjectively assessed as poor, moderate, or good based on body weight, relative size of pectoralis muscles, and relative amount of subcutaneous, epicardial, and coelomic fat (Daoust *et al.* 2021).

Microscopic examination of the skin of some birds revealed small, often oblong structures morphologically compatible with mites. We retrieved intact specimens from the formalin-fixed, ventral skin section of three adult birds with particularly large numbers of them and confirmed that they were mites (Figure 1). A subset of the specimens was prepared by H.P. for slide-mounting. They were first transferred to tap water for several days to leach out the formalin. They were then placed in 80% lactic acid for 24–48 h to clear. Cleared specimens were punctured

FIGURE 1. Numerous deutonymphs of the hypoderatid mite, *Neottialges caparti* Fain, can be seen in the subcutis of a skin sample taken from the mid-ventral region of the abdomen of an adult Northern Gannet (*Morus bassanus*). Most of these nymphs are covered by a thin film of subcutaneous tissue, but are easily visible because of the absence of subcutaneous fat in this emaciated bird. Photo: Jordi Segers.

with a minuten pin, and body contents were gently expressed by squeezing with fine forceps. The mites were mounted on glass slides in commercially prepared polyvinyl alcohol mounting medium (BioQuip Products, Rancho Dominguez, California, USA) and covered with 15-mm round cover slips. Slides were then cured for four days on slide warmers set at 40°C. Mounted mites were examined using a Leica DMLB compound microscope with differential interference contrast (DIC) lighting and photographed using a Leica MC170 HD digital camera and LAS EZ software (Leica Microsystems Canada Inc., Richmond Hill, Ontario, Canada). Photographs were edited using Photoshop v. 23.1.1 (Adobe Inc., San Jose, California, USA).

Results

Necropsies

A section from the ventral region of the skin was examined microscopically in 87 of 313 Northern Gannets (69 adult, five immature, 13 HY). In 31 of these birds, a few to several structures in the subcutis were identified as mites, based mainly on their size and thick body wall (Figure 2). No internal morphology as described by Gardiner and Poynton (1999) for arthropods in tissue sections could be identified. For one additional bird, although no structures compatible with mites were seen in the section from the ventral region of the skin, a structure identified as a mite was seen in the subcutis of a section of leg muscle that had been sampled to examine microscopic details of a traumatic lesion. In three other birds for which a section from the ventral region of the skin was not available, mites were seen in the subcutis of a section of skin taken in proximity to traumatic injuries, from a leg (proximal region) in one instance, and from a wing (distal region) in two instances. In total, we observed mites in 35 of 90 birds whose skin samples were examined microscopically (31 adult, two immature, two HY), indicating a prevalence of 39%.

Although 26 of these 35 birds were considered in poor nutritional condition, in none of them was this thought to be related to the presence of the mites. Based on microscopic examination of the ventral region of the skin, these mites were considered numerous in only five birds, including one in good nutritional condition. In 19 of the 26 birds in poor nutritional condition, an obvious cause, including chronic trauma, was identified. In all cases, inflammation associated with these parasites, which might have represented a drain on the birds' energy reserves, was either absent or minimal, consisting only of small aggregates of macrophages and, in a single case in an adult in good nutritional condition, small granulomas associated with remnants of mites (Figure 3).

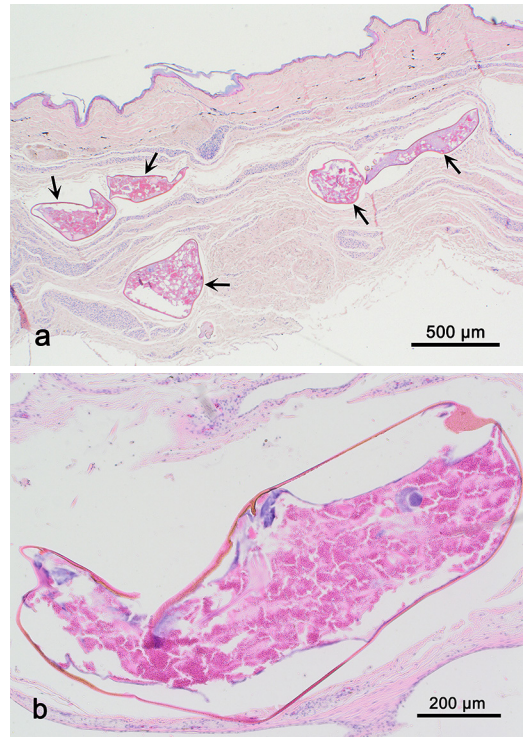

FIGURE 2. a. Several subcutaneous mites (arrows) in a section from the ventral region of the skin of an adult Northern Gannet (*Morus bassanus*). b. Section of a subcutaneous mite at higher magnification, showing the sclerotized cuticle. Hematoxylin and eosin stain. Photos: Pierre-Yves Daoust.

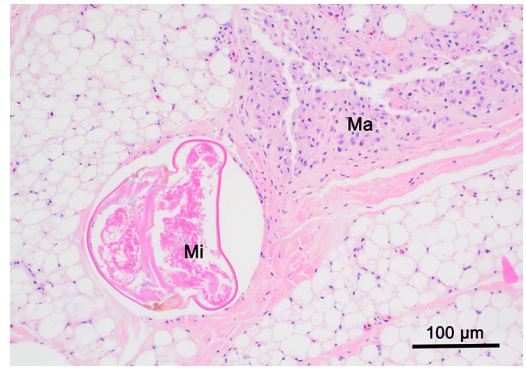

FIGURE 3. Subcutaneous mite (Mi) in a section from the ventral region of the skin of an adult Northern Gannet (*Morus bassanus*). A large aggregate of macrophages (Ma) adjacent to this mite is presumed to represent an inflammatory reaction to its presence. Hematoxylin and eosin stain. Photo: Pierre-Yves Daoust.

Intact mite specimens retrieved from the formalin-fixed subcutis of three adult birds were examined with a stereomicroscope. They had a cylindrical shape

with two pairs of short legs anteriorly and two other pairs in the mid region of the body and had a uniform length of ~1.5 mm (Figure 4). Based on descriptions of small arthropods in a similar location in other species (e.g., Hendrix *et al.* 1987; Pence 2008), we identified them as deutonymphal hypoderatid mites (Acariformes: Astigmata: Hypoderatidae).

Slide-mounted mites

Only five slide-mounted specimens (one to three from each of three birds) had sufficiently complete appendages and setation to be confidently identified using the key and species descriptions in Fain's (1967) key to members of the family Hypoderatidae. All five mites proved to be *Neottialges* (*Pelecanectes*) *caparti* Fain (no common name). Diagnostic features included posteriorly diverging pairs of genital papillae with the posterior papilla larger than the anterior, genital sclerite incomplete and present only anteriorly, tarsus of Leg III with a short hooked tarsal claw, and tarsus of Leg IV with two large spine-like setae at the base of the segment and one small spine-like seta subterminally (Figures 5–7; note that in hypoderatid deutonymphs, tarsus IV terminates in a long whiplike macroseta rather than a tarsal claw). Representative specimens of *N. caparti* were deposited in the New Brunswick Museum, Saint John, NB, Canada (accession nos. NBM-GI-011575, NBM-GI-011576, and NBM-GI-011577) and in the E.H. Strickland Entomological Museum, University of Alberta, Edmonton, Alberta, Canada (accession nos. UASM 80600, 80601, 80602, 80603, and 80604).

Discussion

Approximately 80 species of Hypoderatidae have so far been described, mostly based on the host-associated deutonymphal stage (Mironov and Ramilo 2019). The presence of deutonymphal hypoderatid mites beneath the dermis of birds was reported for the first time by Montagu (1811), who observed them in Northern Gannet from the eastern North Atlantic, although he misidentified them as insects (Fain 1967). Fain (1967) described *Neottialges* (*Pelecanectes*) *bassani* (Montagu) and *N. caparti* co-occurring in two specimens of Northern Gannet from Belgium, whereas Pence *et al.* (1997) later provided a record for *N. bassani* in a single Northern Gannet from Florida, USA. To our knowledge, this is both the first report of *N. caparti* in Northern Gannet from the western North Atlantic and the second report of *N. caparti* since its initial description by Fain (1967) in Belgium. *Neottialges bassani* is separated from *N. caparti* in Fain's (1967) key based on the length of one pair of dorsal setae (*d 4*), which were often missing or broken in our specimens. Given the small number of mites that were morphologically complete enough for us to identify to species ($n = 5$), we cannot rule out that *N. bassani* as well as *N. caparti* are parasites of Northern Gannet in the western North Atlantic.

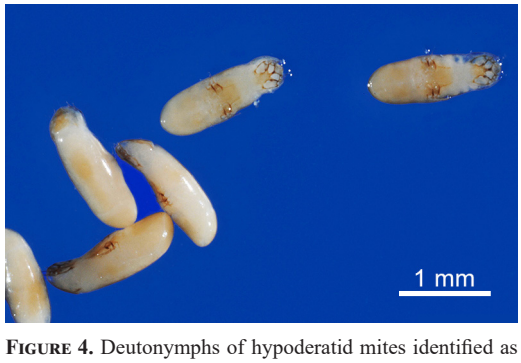

FIGURE 4. Deutonymphs of hypoderatid mites identified as *Neottialges caparti* from an adult Northern Gannet (*Morus bassanus*). Epimerites of the coxal fields are apparent as dark brown lines ventrally. Photo: Shelley Ebbett.

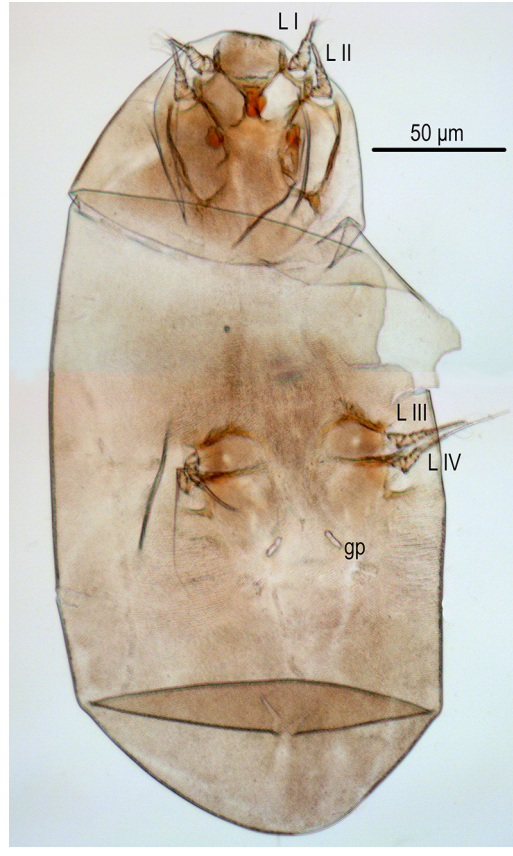

FIGURE 5. Ventral view of cleared, slide-mounted *Neottialges caparti* deutonymph. Labels indicate legs (L I to L IV) and location of genital papillae (gp). Photo: Heather Proctor.

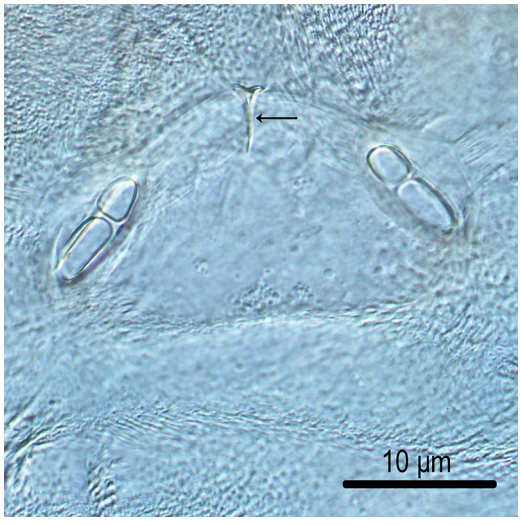

FIGURE 6. Juvenile genitalic area of a *Neottialges caparti* deutonymph showing three features important for diagnosing this species: posterior genital papilla larger than anterior papilla, left and right pairs of genital papillae diverging from each other posteriorly, genital sclerite (arrow) present only in the anterior part of the genital field rather than extending posteriorly between the papillae. Photo: Heather Proctor.

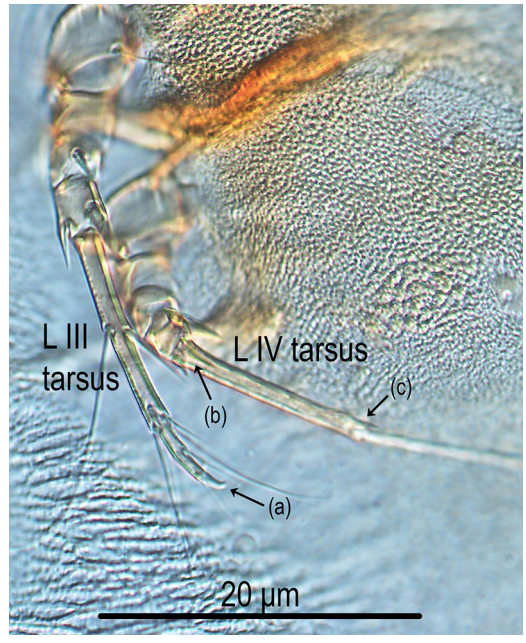

FIGURE 7. Tarsi of legs (L III and L IV) of a *Neottialges caparti* deutonymph. Arrows indicate features important in diagnosing this species: (a) short, curved tarsal claw on L III, (b) restriction of thick spine-like setae to the base of L IV tarsus with (c) one smaller spine-like seta subterminally. Photo: Heather Proctor.

Being very small, of a pale colour, and buried in the host's fat, deutonymphs of hypoderatid mites can easily be missed macroscopically. Yet, they have been reported (Fain 1967; Pence 2008; Mironov and OConnor 2013; Mironov and Ramilo 2019) in avian hosts of most major orders, including Apodiformes (hummingbirds and swifts), Accipitriformes (hawks and eagles), Ciconiiformes (storks), Columbiformes (pigeons), Coraciiformes (rollers), Cuculiformes (cuckoos), Falconiformes (falcons), Gruiformes (cranes), Passeriformes (perching birds), Pelecaniformes (pelicans and relatives), Piciformes (woodpeckers), Psittaciformes (parrots), Strigiformes (owls), and Suliformes (gannets and relatives). Hypoderatid deutonymphs are most commonly observed in the host's subcutis and less often in various other locations in the body, such as around the esophagus and in air sacs and lungs (Fain and Laurence 1974). Northern Gannets, like some other species in the orders Suliformes and Pelecaniformes, have a very elaborate system of subcutaneous air diverticula, most extensive along the ventral region of the chest and abdomen, which communicate bilaterally with the respiratory air sacs in the axillary region (Daoust *et al.* 2008). It was not possible to determine in the present series of cases whether any of the deutonymphs had reached these air diverticula or whether they were all confined to the subcutis. Access to these diverticula could further facilitate the migration of these mites throughout the body.

Our calculated prevalence of 39% is most likely an underestimate because it was based largely on the examination of a single histological section of skin, although the location where most of these sections were taken, in the ventral region of the body midway between the axillary and groin areas, is reported to be a common site for these mites (Fain 1967; Pence 2008). By comparison, Hendrix *et al.* (1987) observed deutonymphal hypoderatids in 21 of 24 (87.5%) adult Cattle Egret (*Bubulcus ibis ibis* (L.)) in Alabama, USA.

To our knowledge, this report is the first to provide information about the demographic distribution of hypoderatid deutonymphs in Northern Gannet. We identified these parasites in roughly the same proportion of adult and immature birds, but in a lower proportion in HY birds. The viability of the deutonymphs at the time of their hosts' death could not be assessed histologically on the basis of the integrity of their internal structure, possibly partly because of autolysis, but also because their internal morphology is normally greatly reduced (Alberti *et al.* 2016). However, the rarity of inflammation and its mild nature when present suggested that these mites were still alive when their hosts died, as dead parasites and their degradation products normally elicit a more intense

inflammatory reaction, as we observed in a single instance.

According to Mironov and Ramilo (2019), the full life-cycle of hypoderatid mites has been described for only four species, and most species are known only from deutonymphs. It is generally understood that, except for the deutonymphs, the various stages of hypoderatid mites live in their hosts' nesting material. The microscopic larvae hatching from the eggs penetrate the intact skin of the birds, presumably of both nestlings and their parents, where they transform into deutonymphs. Following gradual maturation in their host's subcutis, the deutonymphs become activated at nesting time, metamorphose to adults, and exit through their adult host's skin (Pence 2008). The presence of these mites in a least a few HY and immature birds in the present study suggests a long life span within their hosts, because, after fledging, Northern Gannet do not come to land until they reach sexual maturity at 5 years of age. These deutonymphs would need to remain sensitive for that length of time to physiological signals of their host indicative of the nesting season, possibly hormonal as in the classic case of the rabbit flea (*Spilopsyllus cuniculi* (Dale); Rothschild and Ford 1964). The precise nature of these signals is as yet unknown.

Author Contributions

Conceptualization: P.-Y.D.; Data Curation: N.C. and P.-Y.D.; Funding Acquisition: H.C.P. and P.-Y.D.; Investigation: P.-Y.D.; Methodology: H.C.P. and P.-Y.D.; Writing – Original Draft: H.C.P. and P.-Y.D.; Writing – Review & Editing: H.C.P., N.D., and P.-Y.D.

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