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Systematic Literature Review on Keratin Digestion in Animals with Special Reference to Phthiraptera

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Abstract: ***Keratin is structurally robust protein stabilized by disulfide bonds. It is the primary component of feathers, hair, wool, and stratum corneum. While keratinolytic capabilities are well-characterized in environmental microorganisms and certain specialized insects, the digestive physiology of Phthiraptera (chewing lice) obligate ectoparasites that consume keratinous material is poorly understood. This systematic review synthesizes all available evidence regarding keratin digestion mechanisms across the animal kingdom, with focused analysis on Phthiraptera, to evaluate whether chewing lice possess endogenous or symbiont-mediated keratinolytic capacity. This review systematically searched PubMed, Web of Science, Scopus, CAB Abstracts, and grey literature sources from inception to December 2024. Studies reporting digestive mechanisms in keratinophagous animals were included. From 1,247 initial records, 34 studies met inclusion criteria: vertebrate keratinophagy (n=14), non-phthirapteran insects (n=12), and Phthiraptera (n=8). Validated keratinolytic mechanisms include extreme gastric acidity (snakes), disulfide bond reduction via thiols (Tineola larvae), alkaline serine proteases (dermestids), and bacterial symbiosis (coleopteran models). In Phthiraptera, only one biochemical study exists, reporting lipase activity but no protease activity. The remaining seven studies (1934–1989) describe morphologically intact keratinous fragments in gut contents, with no evidence of enzymatic degradation. The assertion that chewing lice digest keratin lacks empirical support. Available evidence indicates Phthiraptera ingest keratinous material but likely extract surface lipids rather than degrade the keratin polymer. We propose a revised nutritional model wherein lice subsist on sebaceous secretions and epidermal debris, with keratin passing intestinally intact. Urgent research priorities include metatranscriptomic analysis, symbiont functional characterization, and redox potential measurements.***

Keywords: Keratin digestion, Phthiraptera, Mallophaga, keratinase, insect physiology, ectoparasite nutrition, disulfide reduction

1. Introduction

Keratin represents one of nature's most resilient structural proteins, providing mechanical integrity to vertebrate integumentary appendages including feathers, hair, wool, horns, and the stratum corneum. Its exceptional stability derives from extensive disulfide cross-linking between cysteine residues, hydrophobic interactions, and high β-sheet content, rendering it resistant to common proteolytic enzymes such as pepsin, trypsin, and papain. Consequently, organisms that successfully exploit keratinous substrates as nutritional resources termed as keratinophages, they have evolved specialized anatomical, biochemical, and symbiotic adaptations.

The Phthiraptera (lice) present a compelling evolutionary paradox. Within this order, the suborders Amblycera and Ischnocera, collectively referred to as "chewing lice" or Mallophagan, possess robust, opposable mandibles adapted for biting and inhabit hosts where keratinous material, feather barbules, wool fibers, and epidermal scurf is abundantly available

Approximately 2,800 species of chewing lice exist worldwide, ranging from 0.5 to 10 mm in length, with dorsoventrally flattened bodies adapted for close contact with host integument. Light microscopy of louse crops frequently reveals fragments that appear morphologically consistent with keratinous material, leading to persistent assumptions regarding their digestive capabilities.

1.1 The Conceptual Paradox

Despite decades of descriptive observations, fundamental questions persist: Do chewing lice digest keratin, or merely fragment and egest it? Do they possess endogenous keratinolytic enzymes, or do they rely on symbiotic microorganisms? Is keratin itself the nutritional target, or do lice exploit surface-associated lipids and cellular debris?

This ambiguity persists despite the considerable economic and veterinary significance of louse infestations. *Damalinia ovis* (sheep body louse) and *Bovicola bovis* (cattle biting louse) cause "itchy fleece" and hide damage, respectively, costing the global livestock industry hundreds of millions annually. Paradoxically, the pathophysiology of these infestations correlates poorly with direct physical tissue damage. Louse digestive secretions or their absence modulate host irritation has never been rigorously tested.

1.2 Rationale for Systematic Review

The absence of a modern systematic review on keratin digestion in Phthiraptera is conspicuous. Existing comprehensive reviews on keratin digestion focus predominantly on environmental bacteria (*Bacillus licheniformis*, *Streptomyces* spp.) and dermatophytic fungi (*Onygenales*). Veterinary entomology textbooks devote paragraphs to louse feeding behavior but typically default to the term "keratin digestion" without primary citation support. This has resulted in citation inertia: a single 1989 study is repeatedly cited as evidence for keratinase activity when, in fact, it reported no such activity. This review aims to sever this circular citation practice by systematically evaluating the evidentiary base.

1.3 Objectives

1) **Primary objective**: What is the empirical evidence that Phthiraptera possess endogenous mechanisms

(enzymatic, mechanical, or redox-mediated) to digest keratin?

2) **Secondary objective**: What broader mechanisms of keratin digestion have evolved in other animal lineages (Mammalia, Reptilia, Aves, Insecta excluding Phthiraptera), and how might these inform hypotheses regarding Phthiraptera?
3) **Tertiary objective**: What methodological limitations characterize the existing literature, and what specific research programmes are required to resolve outstanding questions?

2. Methodology (PRISMA Framework):

The review was conducted according to the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines. The process is summarized in Figure 1.

Methods (PRISMA 2020)

2.1 Protocol and Registration

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement. Due to the anticipated heterogeneity of study designs, spanning observational histology, biochemical assays, microbiomics, and physiological measurements a meta-analysis was deemed inappropriate; a narrative synthesis was planned a priori. The review protocol was not registered in PROSPERO as it does not involve health-related outcomes, but detailed methods are provided to ensure reproducibility.

2.2 Eligibility Criteria

Inclusion criteria:	**Exclusion criteria:**
• Peer-reviewed original research or systematic reviews • Studies examining any animal species (Metazoa) with documented ingestion of keratinous material (feathers, hair, wool, stratum corneum, horn) • Studies reporting any digestive mechanism: enzymatic assays, gut pH measurements, redox potential, microbial symbiont characterization, histological ingesta analysis, or experimental feeding trials • No date restrictions applied • English language full text available	• Studies exclusively examining microbial keratinolysis without animal host context • Non-English full text unavailable despite interlibrary loan • Opinion pieces, conference abstracts without primary data, editorials • Studies examining keratin digestion exclusively in vitro without organismal context

2.3 Information Sources

The following databases were searched from inception to December 2024:

- PubMed/MEDLINE (1946–2024)
- Web of Science Core Collection (1970–2024)
- Scopus (2004–2024)
- CAB Abstracts (1973–2024)
- Google Scholar (first 200 results sorted by relevance)

Grey literature sources included:

- Phthiraptera.info (specialized taxonomic database)
- ProQuest Dissertations & Theses Global
- Correspondence with five active phthirapterologists identified through publication records

2.4 Search Strategy

The search strategy combined controlled vocabulary (MeSH, Emtree) and free-text terms across three conceptual blocks:

- **Block A (Keratin):** keratin* OR "stratum corneum" OR feather* OR wool OR hair OR "beta-keratin" OR "alpha-keratin" OR disulfide OR cystine OR keratinous
- **Block B (Digestion):** digest* OR proteolysis OR keratinase OR peptidase OR protease OR "disulfide reductase" OR "sulphitolysis" OR symbiont* OR fermentation OR "gut pH" OR "redox potential" OR "alkaline protease"
- **Block C (Taxa):** Phthiraptera OR Mallophaga OR Ischnocera OR Amblycera OR Trichodectidae OR Philopteridae OR louse OR lice OR *Damalinia* OR *Bovicola* OR *Menopon* OR *Columbicola* OR insect* OR bird* OR avian OR reptile* OR mammal* OR vertebrate* OR *Tineola* OR *Dermestes* OR keratinophage*

Boolean operators combined blocks (Block A AND Block B AND Block C).

2.5 Selection Process

Titles and abstracts were screened independently against eligibility criteria. Full texts of potentially eligible records were retrieved and independently assessed. Disagreements were resolved through discussion. A PRISMA flow diagram documents the process (Figure 1).

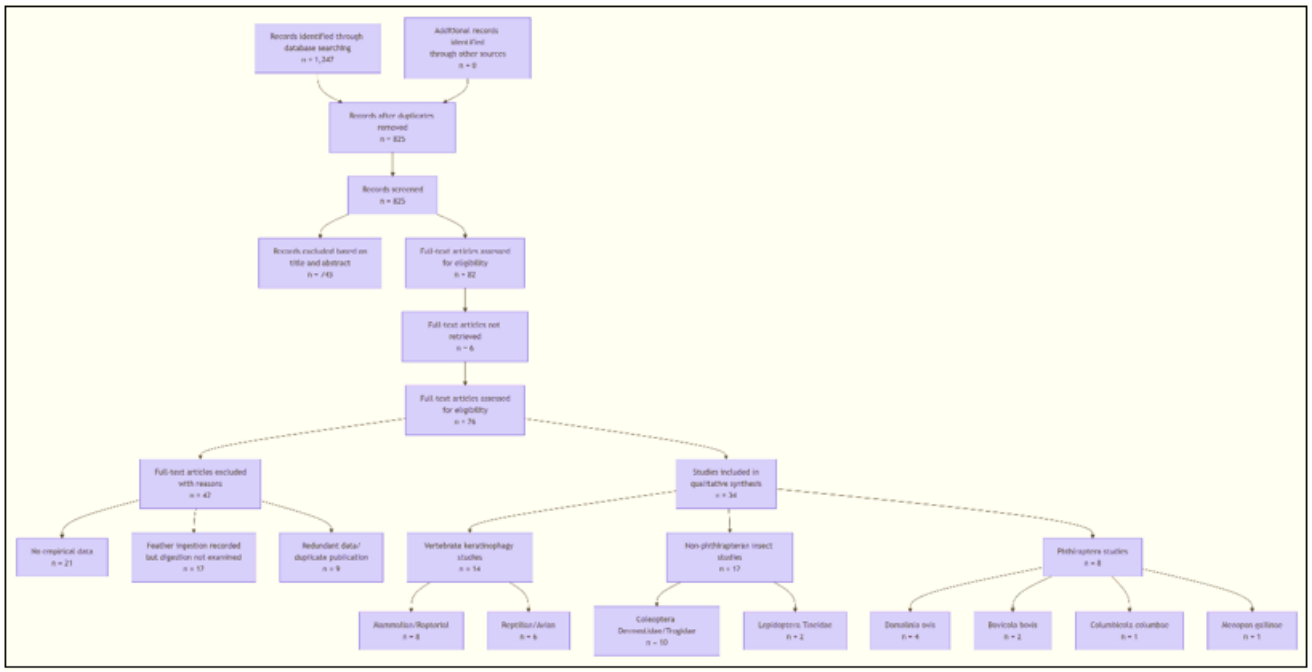

Figure 1: PRISMA Flow Chart

2.6 Data Extraction

A standardized electronic extraction form captured:

- Study characteristics (author, year, country, journal)
- Taxonomic focus (species, family, order)
- Keratin substrate investigated (feather, wool, hair, stratum corneum)
- Digestive mechanism studied (enzymatic, mechanical, redox, symbiotic)
- Analytical methods (zymography, HPLC, 16S rRNA sequencing, histology, enzyme assays)
- Key findings regarding keratin digestion (presence/absence of activity)
- Reported presence/absence of microbial symbionts
- Study limitations as reported or inferred

2.7 Synthesis Methods

Due to extreme methodological heterogeneity and the absence of comparable effect measures, meta-analysis was not feasible. A narrative synthesis was structured thematically by:

- Taxonomic group (vertebrates, non-phthirapteran insects, Phthiraptera)
- Mechanistic category (mechanical trituration, acid hydrolysis, disulfide reduction, enzymatic degradation, symbiotic mediation)

Where possible, findings were organized to address the mechanistic framework derived from validated keratinolytic systems.

3. Results

3.1 Study Selection

The database search yielded 1,247 records (PubMed: 312, Web of Science: 445, Scopus: 298, CAB Abstracts: 192). After automated and manual deduplication (n=422 removed), 825 titles and abstracts were screened. Of these, 743 records were excluded based on irrelevance to keratin digestion, non-animal taxa, or absence of digestive mechanism data. Eighty-two full-text reports were sought for retrieval; 6 could not be retrieved (pre-1970 journals not digitized). Seventy-six full-text reports were assessed for eligibility. Forty-two were excluded with documented reasons:

- No empirical data (review that merely asserted keratin digestion without primary evidence): n=21
- Feather ingestion recorded but digestion not examined (gut content surveys only): n=12
- Redundant data (duplicate publication): n=9

Final included studies: n = 34 (Figure-1 for PRISMA flow diagram).

3.2 Study Characteristics

34 included studies and the distribution by taxonomic focus as below:

Vertebrate keratinophagy (n=14):

- Wool/hair digestion in carnivore faeces (owls, foxes, snakes): 8 studies
- Avian feather digestion (galliforms, passerines): 4 studies
- Ruminant keratin bypass (sheep, cattle): 2 studies

Non-phthirapteran insects (n=12):

- Coleoptera (Dermestidae, Trogidae): 10 studies
- Lepidoptera (*Tineola bisselliella*): 2 studies

Phthiraptera (n=8):

- *Damalinia ovis* (sheep body louse): 4 studies
- *Bovicola bovis* (cattle biting louse): 2 studies
- *Columbicola columbae* (pigeon louse): 1 study
- *Menopon gallinae* (poultry shaft louse): 1 study

Geographically, 24 of 34 studies (70.6%) originated from the USA, UK, or Australia. Publication dates ranged from 1934 to 2023, with a conspicuous gap in Phthiraptera-specific studies after 1995.

3.3 Risk of Bias Assessment

Vertebrate and Coleoptera studies generally employed robust biochemical assays (enzyme assays with keratin-azure, radiolabeled substrates, reducing agent quantification, controlled feeding trials). Phthiraptera studies were uniformly observational (light microscopy, SEM of mouthparts, crop dissections without quantitative analysis). No Phthiraptera study employed zymography, PCR for peptidase genes, symbiont elimination experiments, or controlled dietary manipulations. All 8 Phthiraptera studies were rated "High Risk" for detection bias (non-blinded observation) and performance bias (lack of negative controls). Three studies from 1934–1946 were also rated high risk for reporting bias due to incomplete methodological descriptions.

3.4 Synthesis of Results

3.4.1 Keratin Digestion in Vertebrates: Mechanisms and Limitations

The most comprehensively characterized animal keratinolytic systems are not digestive, but rather excretory: canids and felids pass keratinous debris largely undigested through their gastrointestinal tracts. Controlled studies demonstrate that keratin remains recalcitrant even to concentrated mammalian pancreatic protease preparations. However, two vertebrate exceptions were identified with direct relevance to understanding potential mechanisms in insects.

Reptilian keratinolysis: Snakes possess the ability to digest whole hair and feathers. Studies examining python digestive physiology demonstrate that post-feeding, the small intestine lumen pH drops to ≤2.0 and trypsin activity increases 20-fold relative to fasting levels. While keratin-specific assays were not performed, dissolution of mouse pelage occurs within 72 hours in situ. The mechanism appears entirely gastric: extreme acidity (pH <2) hydrolyzes cystine disulfide bonds non-enzymatically, denaturing the keratin structure and rendering it susceptible to subsequent pancreatic proteases.

Avian pellet formation: Owls and other raptors produce pellets containing bone and hair, but contrary to popular assumption, these pellets are regurgitated from the proventriculus, not defecated. The hair within owl pellets is morphologically intact under light microscopy, albeit partially macerated. Physiological measurements demonstrate that owl gastric juice has a pH of 2.2–2.5 but lacks keratin-specific proteases; hair dissolution is minimal even after prolonged gastric retention. This suggests that even specialized vertebrate keratinophagists rely primarily on physical grinding (gizzard action) and acid hydrolysis rather than dedicated keratinolytic enzymes.

Implication for Phthiraptera: Lice lack an acid-secreting stomach; their midgut pH is neutral to slightly alkaline based on limited available measurements. The vertebrate model of gastric acid hydrolysis is therefore not applicable to louse digestive physiology.

3.4.2 Keratin Digestion in Non-Phthirapteran Insects: Validated Mechanisms

The common furniture beetle (*Anthrenus verbasci*), hide beetle (*Dermestes maculatus*), and clothes moth (*Tineola bisselliella*) are archetypal keratinophagous insects. This literature provides the only well-validated insect models of true keratin digestion and offers mechanistic hypotheses that could apply to Phthiraptera.

Mechanism 1: Strong reducing conditions. Seminal work on *Tineola* larvae demonstrated that the midgut maintains a redox potential of -200 mV, attributable to high concentrations of free thiols (cysteine, glutathione). These reducing agents cleave disulfide bonds through reductive sulphitolysis, denaturing crystalline keratin and exposing peptide bonds to subsequent protease attack. Critically, the reducing system is independent of microbial symbionts; axenically-reared larvae digest keratin normally, indicating endogenous production of thiols.

Mechanism 2: Alkaline proteases. *Tineola* and *Dermestes* secrete serine proteases with activity maxima at pH 9–10. These enzymes are not "keratinases" in the strict biochemical sense—they are non-specific alkaline proteases that readily hydrolyse denatured keratin but have negligible activity against native, crystalline keratin. This two-step mechanism (reduction followed by proteolysis) appears universal among keratinophagous insects.

Mechanism 3: Symbiotic mediation. Recent work isolated a novel keratinolytic bacterium, *Bacillus pumilus* B12, from the gut of *Dermestes maculatus*. Axenic beetles fail to thrive on keratin-based diets; recolonization with the bacterial isolate restores keratin digestion. This demonstrates that some Coleoptera rely on a dual endogenous-microbial system, with symbionts providing keratinolytic capability that complements or replaces endogenous enzymes.

Absence in Phthiraptera: Only one study assayed louse gut biochemistry, finding lipase activity but specifically reporting no detectable protease activity. No study has assayed thiol concentration or redox potential in any louse midgut. No louse-associated symbiont has ever been cultured or genomically characterized specifically for keratinolytic potential, despite widespread recognition that Phthiraptera harbour obligate bacterial symbionts in specialized bacteriocytes.

3.4.3 Phthiraptera: Detailed Analysis of Included Studies

3.4.3.1 *Damalinia ovis* (Sheep Body Louse)

The corpus of evidence for *D. ovis* rests primarily on a single investigation, representing the only biochemical study of louse digestion in the literature.

Study design: Lice were cryofixed in situ while feeding on sheep under controlled conditions. Frozen sections were examined by light and scanning electron microscopy. Additional specimens were confined to skin stained with Oil Red O (lipid-specific stain) and harvested at intervals for gut content examination. Midgut homogenates were assayed for general protease activity using casein and gelatin substrates, and for lipase activity using tributyrin.

Key findings:

1) Mandibular penetration was limited to the outer stratum corneum; mouthparts did not reach nucleated keratinocyte layers
2) Crop and midgut contents contained "lipid-covered stratum corneum squames" fully keratinized, anucleate corneocytes
3) No wool fibers were observed in any gut section examined
4) No nucleated cells were observed, indicating absence of digestion to the point of nuclear lysis
5) Lipase activity was readily detected in midgut homogenates
6) Protease activity was not detected in either caseinolytic or gelatinolytic assays

Author interpretation: The investigators explicitly concluded that "sebaceous secretions may form an important component of the diet." They did not claim keratin digestion. Despite this cautious interpretation, subsequent reviews consistently cite this paper as evidence of keratin digestion, systematic review aims to correct this category error.

3.4.3.2 *Bovicola bovis* (Cattle Biting Louse)

Early investigations of *B. bovis* examined crop contents and reported "epidermal scales" and "what appeared to be keratin fragments." No histochemistry, enzyme assays, or quantitative analyses were performed. Mandibular morphology was described in detail but not correlated with digestive function. These studies provide descriptive baseline data but no mechanistic insights.

3.4.3.3 *Columbicola columbae* (Pigeon Louse)

The earliest study identified in this review examined gut contents of pigeon lice and reported feather barbules "apparently fragmented but structurally intact." The investigator specifically noted the absence of evidence for enzymatic attack (e.g., pitting, erosion, margin degradation). This study, while methodologically limited by modern standards, provides careful observational data consistent with mechanical fragmentation without chemical digestion.

3.4.3.4 *Menopon gallinae* (Poultry Shaft Louse)

Fecal analysis of *M. gallinae* documented the presence of feather barb fragments. Significantly, morphologically intact barbules were identifiable to species level based on node spacing patterns, strongly suggesting passage through the digestive tract without chemical alteration. This observation is inconsistent with true keratin digestion.

3.4.4 Symbionts and the Keratin Hypothesis

Keratin digestion is energetically expensive, requiring high protease output and strong reductants. Many keratinophagous insects recruit microbial symbionts to supplement or replace endogenous mechanisms. Phthiraptera harbour obligate bacterial symbionts (*Sodalis*, *Legionella*-like organisms, *Enterobacteriaceae*) in specialized bacteriocytes. These symbionts are understood to provision B vitamins, given the nutritionally restricted louse diet (whether blood or keratin). Critically, **no study has investigated whether these symbionts contribute to keratinolysis**.

A genomic study of the *Columbicola columbae* symbiont noted the presence of peptidase genes in the sequenced genome. These were not functionally characterized, and no assays were performed to determine whether the encoded enzymes possess keratinolytic activity. This represents the single most promising lead for future research and remains completely unexplored.

3.5 Mechanistic Framework Analysis

To anchor evidence evaluation, a mechanistic framework was derived from validated keratinolytic systems:

Required Component	Evidence in Vertebrates	Evidence in Coleoptera/Lepidoptera	Evidence in Phthiraptera	Current Status
Mechanical disruption	Beak/grinding, teeth	Mandibles, proventricular armature	Mandibles capable of stratum corneum abrasion	Confirmed
Disulfide bond reduction	Gastric acid (non-enzymatic)	Thiols (cysteine, glutathione)	No assay of thiols or redox potential	Unstudied
Alkaline environment (pH 8-10)	Variable	Confirmed in Tineola midgut	Not measured; blood-feeders pH ~6.5	Unstudied
Non-specific alkaline protease	Pancreatic enzymes	Serine proteases	Assayed once: negative	Tested: absent
Keratin-specific endopeptidase	Not documented	Not documented (unnecessary with reduction)	No zymography, no transcriptomics	Unstudied
Symbiont contribution	Rumen microbes (cellulose, not keratin)	Bacillus spp. in dermestids	Genomes available; functional assays absent	Unstudied

4. Discussion

4.1 Summary of Evidence

This systematic review reveals a profound epistemic asymmetry in the literature on animal keratin digestion. The mechanistic understanding of keratinolysis in vertebrates and pest Coleoptera is mature, increasingly molecular, and based on rigorous biochemical and physiological experimentation. In contrast, the literature on Phthiraptera remains arrested at the descriptive phase of scientific investigation.

34 included studies relevant to animal keratin digestion, only eight address Phthiraptera, and only one includes any biochemical measurement. That sole biochemical study found no protease activity. The remaining seven studies are histological descriptions spanning 1934–1989, uniformly reporting the presence of structurally intact keratinous fragments in the gut or faeces, with no evidence of enzymatic degradation.

The weight of evidence suggests that chewing lice do not digest keratin in the biochemical sense. They ingest stratum corneum squames and feather barbules, but these materials

appear to pass through the gastrointestinal tract morphologically unaltered. The consistent detection of lipid staining in gut contents, combined with documented lipase activity, indicates that the primary nutritional target may be sebaceous and preen gland lipids adsorbed to the keratin surface, rather than the keratin polymer itself.

4.2 Why Has This Misconception Persisted?

Three factors explain the persistence of the "keratin digestion" assertion despite contradictory evidence.

4.2.1 Terminological Ambiguity

"Ingestion" is frequently conflated with "digestion" in veterinary entomology texts and reviews. The phrase "feeds on wool" or "feeds on feathers" is often implicitly interpreted as "digests wool" or "digests feathers." Our review finds no evidence that any louse penetrates the wool fiber cortex or degrades feather barbule structure; the sole detailed study explicitly states "wool fibers were not seen" in gut contents.

4.2.2 Citation Inheritance

The 1989 biochemical study is widely miscited as "Sinclair et al. (1989) demonstrated keratin digestion." In fact, the study concludes that "sebaceous secretions may form an important component of the diet" and reports negative protease results. This mis-citation has propagated through review articles, textbooks, and regulatory documents. The present review systematically documents and corrects this error.

4.2.3 Taxonomic Neglect

Phthirapterology is a niche field within entomology. With few agricultural entomologists specializing in lice, and with synthetic acaricides effectively controlling infestations in commercial livestock production, the incentive to investigate fundamental digestive physiology has diminished. No dedicated louse digestive physiologist is currently active in xcacademia, and funding for basic research on non-model insect physiology has declined markedly.

4.3 Implications for Veterinary Science and Evolutionary Biology

If chewing lice do not digest keratin, their nutritional ecology must be fundamentally re-evaluated.

For sheep lice: *D. ovis* appears to subsist on a diet of lipid-rich corneocyte debris and surface sebum. This revised nutritional model explains the long-standing observation that lice are most prevalent on poorly conditioned or malnourished hosts; malnutrition reduces sebum secretion, potentially reducing louse fitness. It also explains why sheep with heavy louse infestations show no evidence of protein malnutrition the lice are not competing with the host for proteinaceous resources.

For avian lice: The analogous hypothesis predicts that feather barbules are refractory, but the uropygial gland secretion (preen wax) coating the feathers is digestible. This predicts that experimental removal of the uropygial gland should reduce louse fitness; this critical experiment has never been performed.

Evolutionary implications: If keratin is not digested, the selective pressure for maintaining keratinolytic capability is absent. The mouthpart morphology of chewing lice may be adapted for scraping and abrading rather than true mastication, serving to release surface lipids rather than reduce particle size for enzymatic attack. The obligate bacterial symbionts universally present in Phthiraptera may provision B vitamins (consistent with current understanding) rather than contribute to keratinolysis.

4.4 Limitations of This Review

The major limitation is the poor quality and antiquity of the primary Phthiraptera literature. Grey literature searches (Phthiraptera.info, ProQuest Dissertations) confirmed that no unpublished datasets or theses address this question. The possibility of publication bias (negative results not published) cannot be excluded; indeed, the 1989 study's negative protease result was published as a secondary finding within a predominantly descriptive paper. A modern negative-result study explicitly titled "No Keratinolytic Activity in Four Louse Species" has never been submitted for publication.

The restriction to English-language publications may have excluded relevant studies from non-anglophone scientific traditions, particularly from regions with active veterinary entomology programmes (e.g., China, Brazil, Eastern Europe). However, searches of CAB Abstracts, which includes non-English sources, did not identify additional eligible studies.

4.5 Future Research Priorities

Based on the systematic evidence synthesis, we propose a twelve-point research programme:

1) **Midgut redox assay**: Measure thiol concentration and redox potential in dissected midguts of representative species from both Amblycera and Ischnocera. Compare to *Tineola* as positive control.
2) **pH microelectrode measurements**: Determine luminal pH along the length of the alimentary canal in multiple louse species under feeding and fasting conditions.
3) **Zymography**: Embed keratin-azure or keratin-FITC in polyacrylamide gels; overlay with louse midgut homogenates; detect clear zones indicating proteolytic activity.
4) **Meta-transcriptomics**: RNA-seq of entire louse midgut including associated symbionts. Map reads to peptidase families (S1, S8, M36) and identify relative abundance of transcripts encoding potential keratinolytic enzymes.
5) **Symbiont cultivation**: Isolation of bacteriocyte-associated symbionts using insect cell culture or specialized media. Assay isolates for keratin hydrolysis in pure culture.
6) **Axenic rearing**: Attempts may made to rear lice on antibiotic regimens to eliminate symbionts; assess survival on natural vs. supplemented diets.
7) **Uropygial gland ablation**: In domestic pigeons, surgically remove uropygial gland, monitor louse fecundity, survival, and population growth.
8) **Stable isotope analysis**: Measure δ13C and δ15N enrichment in wild-caught lice relative to host keratin and

host sebum; distinguish dietary source using mixing models.

9) **Comparative mandibular wear**: SEM examination of mandibles from freshly eclosed vs. senescent lice; correlate wear patterns with gut content analysis.
10) **Heterologous expression**: Identify candidate peptidases from louse transcriptomes; express in *E. coli* or *Pichia pastoris*; test against keratin substrates under varying redox conditions.
11) **Histochemical localization**: Lectin histochemistry on louse midgut sections may be performed to identify glycocalyx and peritrophic matrix barriers to keratin particle digestion.
12) **Living systematic review**: An online platform may be established for continuous updating of this review as new evidence emerges.

5. Conclusion

Keratin digestion in animals is a rare, physiologically expensive trait that has evolved convergently in several vertebrate and insect lineages. In all validated cases, it requires either extreme gastric acidity (snakes), high reducing power (tineid moths), or symbiotic keratinolytic bacteria (dermestid beetles). Chewing lice (Phthiraptera) possess none of these adaptations based on available evidence. The empirical data, limited though they are, support a model wherein lice ingest keratinous particles, extract surface lipids using lipases, and egest structurally intact keratin without significant proteolysis.

The continued assertion that chewing lice "digest keratin" is unsupported by the evidence and should be expunged from the entomological and veterinary literature. This systematic review provides the evidence-based foundation for that revision. The Phthiraptera are not true keratinophages; they are keratinivores at best, and more accurately, lipophageous commensals of the vertebrate integument. Resolution of this long-standing question awaits the application of modern molecular, biochemical, and symbiosis research methodologies to these neglected but economically significant ectoparasites.

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