Epizootic and zoonotic helminths of the bobcat (Lynx rufus) in Illinois and a comparison of its helminth component communities across the American Midwest

Shelby J. Hiestand1,2, Clayton K. Nielsen2,3, and F. Agustín Jiménez1,*

1 Department of Zoology, Southern Illinois University, Carbondale, Illinois 62901-6501, USA
2 Cooperative Wildlife Research Laboratory, Southern Illinois University, Carbondale, Illinois 62901, USA
3 Department of Forestry and Center for Ecology, Southern Illinois University, Carbondale, Illinois 62901-4619, USA

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Abstract – A total of 6257 helminths of 19 taxa were recovered from the digestive tract and lungs of 67 bobcats in Illinois. Infections caused by Alaria mustelae, Diphyllobothrium latum, and Macracanthorhynchus ingens are reported for the first time in bobcats. From all the taxa recovered, only three species occurred in high prevalence and caused intense infections: Taenia rileyi, Alaria marcianae, and Toxocara cati, with prevalence and mean intensity of 70% and 6; 42% and 193, and 25% and 14 individuals, respectively. Prevalence lower than 15% of 14 helminth species suggests bobcats are not continuously exposed to infective stages of a single parasite, and may be exposed to a large variety of generalists during their lifespan. No significant difference in parasite species according to host sex or age was detected, except for Diphyllobothrium spp., which were found more frequently in females and in trapped bobcats, and the hookworm, Ancylostoma caninum, which infected juveniles more frequently. Average species richness per infracommunity was 2.4 (±1.2), and the parasite component community showed low qualitative similarity with neighbor communities. The taxa A. caninum, Alaria spp., Diphyllobothrium spp., Paragonimus kellicotti, and T. cati are etiological agents of epizootic and zoonotic diseases.

Key words: Epizootic, Zoonotic helminths, Carnivore, Lynx rufus, Bobcat, Diphyllobothrium latum.

Résumé – Helminthes épizootiques et zoonitiques du lynx roux (Lynx rufus) en Illinois et une comparaison de ses communautés de parasites dans le Mid-Ouest Américain. Un total de 6257 helminthes de 19 taxa ont été collectés de l’appareil digestif et des poumons de 67 lynx roux dans l’Illinois. Les infections causées par Alaria mustelae, Diphyllobothrium latum et Macracanthorhynchus ingens sont rapportées pour la première fois chez les lynx roux. De tous les taxa collectés, seules trois espèces présentaient une prévalence importante et causaient des infections intenses : Taenia rileyi, Alaria marcianae et Toxocara cati, avec des prévalences et intensités moyennes de respectivement 70 % et 6 individus, 42 % et 193 et 25 % et 14. Les prévalences inférieures à 15 % de 14 espèces d’helminthes suggèrent que les lynx roux ne sont pas exposés à des parasites stades infectieux d’un seul parasite unique, et peuvent être exposés à une grande variété de généralistes au cours de leur vie. Aucune différence significative dans les espèces de parasites selon sexe et l’âge n’a été détectée, sauf pour Diphyllobothrium spp., qui a été trouvé plus fréquemment chez les femelles et chez les lynx piégés, et l’ankylostome Ancylostoma caninum, qui infectait plus souvent les juveniles. La richesse en espèces moyenne par infracommunauté était de 2.4 (±1.2), et la communauté des parasites a montré une faible similarité qualitative avec les communautés voisines. Les taxa A. caninum, Alaria spp., Diphyllobothrium spp., Paragonimus kellicotti et T. cati sont des agents étiologiques de maladies épizootiques et de zoonoses.

Introduction

In the United States, the top five neglected parasitic infections include four zoonotic diseases, namely Chagas disease, cysticercosis, toxocariasis, and toxoplasmosis [4]. Excluding cysticercosis, the etiological agents of these diseases include parasites that infect domestic and wild felids [7, 10, 21, 65]. Bobcats (Lynx rufus) are the most abundant and widely distributed wild felid species in North America. They range from...
southern Canada to the northern half of Mexico and are nearly ubiquitous throughout the US, including areas of high human density [23, 24]. Highly agricultural areas of the Midwest including locations in Illinois, Indiana, and Ohio provide relatively poor habitat for bobcats [42, 71]. However, hunting and trapping regulations have allowed populations to rebound from overharvesting; bobcats have been protected from legal harvest in Illinois since 1971 and were listed as threatened in the state until 1999 when they were down-listed to non-game species status [41, 43]. Bobcat population densities were estimated to be 0.27/km² in the late 1990s and have increased at a rate >6% since then [41]. The increase in bobcat populations is considered a success in wildlife management [53], yet it also raises concerns about the proportional increase in their role as reservoirs of epizootic and zoonotic parasites [5, 51, 64]. Currently, bobcat abundance is stable or increasing in every state except Florida and Delaware, with a population estimate of about 2.3 million individuals in the US [53].

Parasites of bobcats have been surveyed in bordering regions of Canada and Mexico, as well as in 14 states in the US, including characterization of infections in populations from Arkansas, Georgia, Massachusetts, Nebraska, North and South Carolina, Texas, Virginia, and West Virginia [26, 50, 57, 58, 61, 67, 69, 73]. Each population appears to harbor a unique community of parasites that appear to be a function of the habitat, prey availability, and climate of the region [67, 69, 73]. The differences among communities appear to be congruent with the expectation of an inverse correlation between the similarity of helminth communities and geographic distance in populations of vertebrates [31, 45, 47].

Among the parasites that infect bobcats, three taxa of protozoans and five of helminths are epizootic, and therefore are shared with other carnivores including coyotes, cougars, and domestic cats and dogs; these include *Cytauxzoon felis*, *Diphyllolobothrium mansonioides*, *Echinococcus oligarthrus*, *Mesocestoides variabilis*, *P. kellicotti*, *Sarcozystis spp.*, *Taenia spp.*, and *Toxoplasma gondii*. Six known species of parasites reported in bobcats are known to be agents of zoonotic disease including *P. kellicotti*, *E. oligarthrus*, *D. mansonioides*, *Dirofilaria striata*, *Toxocara cati*, and *Toxoplasma gondii* [10, 72]. The prevalence of these and other etiological agents of zoonotic disease are higher in areas where bobcats, cougars, and domestic cats occur in sympatry [5].

Given the potential for cougars to recolonize the Midwest [34], the high density of bobcats [53, 74] and domestic cats [37], and the continued expansion of human populations [12], it is necessary to document and archive the parasites infecting bobcats. The objective of the present study is to characterize the helminth component community in an area of the Southeast Temperate Plains near an agricultural zone and compare it against helminth component communities from the same ecological region (Georgia, North and South Carolina, Virginia, and West Virginia), the Southeast Coastal Plains (New England), the Ozark/Ouachita Forest (Arkansas), the Semiarid Plains (Nebraska and Texas), and Mixed Woods (Minnesota). To complete this goal, we also characterize infections in bobcats, compare the effect of age and sex on infections, and establish the qualitative similarities of bobcat helminth communities across their distributional range.

### Materials and methods

#### Study area

This survey was completed in the 39 southernmost counties in Illinois (46,436 km²) and only 1 bobcat was obtained from the northern part of the state (Figure 1). The population of bobcats in southern Illinois resides at the edge of areas considered as highly suitable habitat (forested regions) and maize monoculture, generally considered poor habitat for these felines. The southern region of Illinois is human-dominated (ca. 21.5 persons/km²) and comprises the southern till plain, Wabash border, Shawnee hills, Ozark, lower Mississippi river bottomlands, and coastal plain natural divisions [40, 62]. Streams and roads are abundant in the landscape. Land cover consists primarily of closed-canopy mixed hardwood forests (44%; *Quercus* and *Carya* spp.), grasslands (21%), and cropland (19%), [38]. Wetlands (8%), open water (6%), and urban (2%) cover types comprise the remainder of the study area [2].

#### Parasitological examination

Sixty-seven bobcats (52 adults, 15 juveniles) were collected between July 2003 and August 2012. Most of the individuals (96%) were collected in the period between 2010 and 2012. The total included 34 males (27 adults and 7 juveniles) and 33 females (25 adults and 8 juveniles). All animals were in fair to good condition with adults weighing from 5.0 to 17.3 kg (males) and 5.5–10.4 kg (females). The sample included 46 road killed bobcats and 21 individuals that were trapped for the project. From the latter set, 13 individuals were captured by licensed trappers seeking other species.

Specimens were weighed, sexed, and either immediately processed or frozen at −20 °C until necropsy. All methods were supported by the Institutional Animal Care and Use Committee of Southern Illinois University (Assurance Number A-3078-01). Age was determined using the presence of annuli in canine cementum layers of a lower canine tooth [15]. Bobcat ages were grouped into three distinct categories: young of year (YOY) (<1 year), juvenile (1–2 years), and adult (>3 years). These age categories are commonly used in the characterization and comparison of infections in carnivores [25, 30].

The helminthological examination included inspection of the body cavity and internal organs. All organs were prospected for helminths including the heart, lung, liver, stomach, and lower intestinal tract. All macroscopically evident parasites were separated into Petri dishes containing saline solution or tap water and were fixed and preserved immediately. Organ contents were sieved through 350- and 500-μm mesh. The filtered materials were processed by sedimentation and the sediment was examined to recuperate all parasites [20].

Parasites were preserved in a solution of 70% ethanol or formaldehyde; live worms were relaxed by inducing osmotic shock or submerged in glacial acetic acid. Subsequently, worms were fixed as described above. Platyhelminthes were stained in Semichon’s acetic carmine prior to mounting in Canada balsam. Identification of the parasites was completed using specialized literature [11, 17, 36, 52, 68]. Voucher specimens were
deposited in the United States National Parasite Collection (USNPC, Bethesda, Maryland).

Data analysis

Ecological definitions of abundance, intensity, prevalence, and communities follow Bush et al. [9]. Simpson’s diversity index was calculated to measure the concentration of dominance of helminth species [29]. Values of intensity and species richness were first tested for normality ($\alpha = 0.05$ throughout) and then transformed using the logarithm of the original value plus 1. To test for significant differences among parasite communities in roadkills and trapped animals, normalized data of intensity of each parasite species and species richness were tested using a Student $t$-test. The same test was used to compare species intensity and species richness depending on sex. ANOVA was used for test differences among the three age groups. Data that could not be transformed were analyzed using non-parametric Kruskal-Wallis tests for age and sex. The Pearson Chi-square test with a correction for continuity for samples sizes <200 was used to compare expected with observed values of frequency of occurrence for pairs of helminth species [66]. The Chi-square test was also used to determine significant associations between species richness and intensity of species with either host age or sex.

Gastrointestinal helminth component communities were compared against similar bobcat parasite surveys with sample sizes >30 individuals, with the exception of communities from Arkansas, which was based on 10 individuals [26]. The expectation was to find clustering of these communities dependent on five ecoregions including: the Southeast Temperate Plains (Illinois, Georgia, North and South Carolina, Virginia, and West Virginia), the Southeast Coastal Plains (New England), the Ozark/Ouachita Forest (Arkansas), the Semiarid Plains (Nebraska and Texas), and Mixed Woods (Minnesota) [39, 49, 55, 67, 69, 73]. These ecoregions follow the definitions provided by the Environmental Protection Agency [3]. Qualitative similarity of these parasite communities was estimated by means of Jaccard’s similarity index using SAS [59].

As a generalist mesopredator the bobcat is expected to accumulate a large number of helminth species. To visualize this trend a rarefaction curve was constructed based on a sample-based approach, using the program EstimateS 9.1.0 [13]. This curve was constructed based on the expected richness in the pooled sample of infracommunities based on the empirical dataset. Curves for the lower and upper 95% confidence intervals were also constructed. All estimates were made at 67 nodes, replicating the original number of observations, and are based on 100 iterations, with sampling at each node and replacement of individuals between replicates.

Results

A total of 6257 helminths of 18 species, including 6 cestodes, 4 digeneans, 7 nematodes, and one acanthocephalan were recovered (Table 1). Internal organs positive for infection included the stomach, small intestine, and lungs. From that number, a single species of nematode was recovered from the stomach, whereas 14 species were found in the small intestine (three digeneans, six tapeworms, four nematodes, and one acanthocephalan). Three species were recovered from the lungs, including one digenean and two nematodes. Only one bobcat

Figure 1. Collection locations for trapped (13) or road killed (32) individuals used to characterize infections suffered by bobcats (Lynx rufus) in Illinois during 2003–2012. States where other surveys have been completed are shaded gray.
was free of helminth infection. Mean species richness in infra-
communities was 2.4 (±1.2 SD) with a range from 0 to 6. Simp-
son’s index was relatively low (x = 0.13). Both lower and
upper 95% confidence intervals of the estimated species rich-
ness (S) do not converge with the expected S. The latter curve
does not reach a plateau (Figure 2). No significant differences
were found with respect to collection method and as a result,
all roadkills and trapped bobcats were pooled. There were no
differences in species intensity between males and females,
and only Diphyllobothrium spp. were found more commonly
in females (v2,1,67 = 4.45, p = 0.04) and trapped animals
(v2,1,67 = 6.12, p = 0.01). The dog hookworm, A. caninum,
was common in young animals and never found in adults
(v2,2,67 = 6.24, p = 0.04). Concomitantly, A. caninum was present
at higher intensities in YOY and juvenile bobcats when compared with adults (F1,5 = 8.20, p = 0.04). No significant
differences were found with respect to parasite intensity for
sex or age class. There were no significant differences of prev-
ance according to sex or age class.

The comparison of parasite communities (Table 2) resulted
in low similarity among all regions (Figure 3). The distance
between clusters was >0.5 for all locations. The most similar
parasite communities include pairs in Georgia and West
Virginia (0.56), and Nebraska and Texas (0.53). The communities
with the greatest similarity with southern Illinois were those
in North and South Carolina (0.33). The most dissimilar were
Arkansas to Texas (0.05) and New England to Arkansas
(0.08). The area accumulation curve shows non-convergence
between the estimated number of species and their confidence
intervals.

Discussion

Helminths infecting bobcats include a large number of generalist species; with the exception of D. latum, A. mustelae,
and M. ingens, all observed species have been previously reported
from bobcats elsewhere in their range. All 19 taxa varied in
prevalence, intensity, and abundance, although 15 occurred
with prevalence lower than 10%. Parasites of relatively high
prevalence included T. rileyi (70%), A. marcianae (42%),
and T. cati (25%); these species have been found in other bobcat-
dwelling helminth surveys and are considered either bobcat-
specific, such as T. rileyi, or feline-specific, such as T. cati
[26, 39, 49, 55, 67, 69, 73]. Four taxa contain known agents
of zoonotic disease, including Alaria spp., Diphyllobothrium
spp., T. rileyi, and Toxocara cati [16, 18, 28, 44, 63]. Four taxa contain known agents
of zoonotic disease, including Alaria spp., Diphyllobothrium
spp., T. rileyi, and Toxocara cati [16, 18, 28, 44, 63]. The majority of parasite species found are epizootic, in that they may use other species including coyotes, red and grey fox (Vul-
pes vulpes, Urocyon cinereoargenteus), and domestic dogs and
cats (C. lupus familiaris, Felis catus) as alternate definitive
hosts [54]. Their presence in bobcats may be the result of the
generalist nature of these parasites and the opportunistic diet
of bobcats [46]; this diet includes lagomorphs, rodents, white-
tailed deer, and birds as common prey species [35, 70, 74].

| Table 1. Characterization of helminth infections from bobcats, Lynx rufus, in Illinois, based on 67 individuals. Collection numbers from the United States National Parasite Collection are included. |
|---------------------------------------------------------------|
| **Prevalence (%)** | **Mean abundance, ±SD** | **Intensity** | **USNPC No.** |
| **Range** | **Mean, ±SD** | |
| **Trematoda** | | | |
| Alaria americana (I) | 5 | 0(±3) | 1–22 | 8(±12) | 106440 |
| Alaria marcianae (I) | 42 | 81(±385) | 1–2872 | 193(±547) | 106441 |
| Alaria mustelae (I) | 5 | 0(±1) | 2–4 | 3(±1) | 106710 |
| Paragonimus kelticott (L) | 6 | 0(±2) | 1–16 | 5(±7) | 106450 |
| **Cestoda** | | | | |
| Diphyllobothrium mansonoides (I) | 9 | 1(±5) | 1–33 | 11(±13) | 106708 |
| Diphyllobothrium latum (I) | 6 | 0(±1) | 1–3 | 2(±1) | 106707 |
| Mesocestoides sp. (I) | 2 | 0(±4) | 31 | 31 | 106706 |
| Taenia pseudolatilicollis (I) | 5 | 1(±4) | 1–31 | 11(±17) | 106445, 106709 |
| Taenia pisiformis (I) | 5 | 0(±2) | 1–10 | 7(±5) | 106444 |
| Taenia rileyi (I) | 70 | 4(±4) | 1–18 | 6(±4) | 106446, 106447 |
| **Nematoda** | | | | |
| Anaglaroides rostratus (L) | 8 | 0(±1) | 1–7 | 3(±2) | 106716 |
| Ancylostoma caninum (I) | 9 | 1(±3) | 1–18 | 6(±7) | 106713 |
| Molineus barbatus (I) | 14 | 1(±4) | 1–26 | 8(±10) | 106712 |
| Physaloptera sp. (S) | 2 | 0(±0) | 1 | 1 | 106714 |
| Toxascaris leonina (I) | 6 | 0(±1) | 1–6 | 3(±2) | 106452 |
| Toxocara cati (I) | 25 | 3(±11) | 1–60 | 1(±18) | 106451 |
| Vogelioides felis (L) | 6 | 0(±0) | 1–2 | 2(±1) | 106715 |
| Ascarid juveniles (I) | 20 | 1(±3) | 1–16 | 4(±5) | 106711 |
| **Acanthocephala** | | | | |
| Macracanthorhynchus ingens (I) | 2 | 0(±0) | 1 | 1 | 106711 |

(I) Small intestine, (L) Lung, (S) Stomach.
Table 2. Gastrointestinal helminths recorded in the bobcat, *Lynx rufus*.

| Locality                  | Species                                      |
|---------------------------|----------------------------------------------|
| Arkansas (n = 10)         | *Ancylostoma caninum*, *Dipylidium caninum* |
| Georgia (n = 50)          | *Ancylostoma reconditum*, *Toxascaris leonina* |
| Minnesota (n = 75)        | *Ancylostoma caninum*, *Dipylidium caninum* |
| N. & S. Carolina (n = 120)| *Ancylostoma caninum*, *Dipylidium caninum* |
| Illinois (n = 100)        | *Dipylidium caninum*, *Toxascaris leonina*   |
| Nebraska (n = 143)        | *Ancylostoma caninum*, *Dipylidium caninum* |
| Texas (n = 66)            | *Ancylostoma caninum*, *Dipylidium caninum* |
| West Virginia (n = 80)    | *Ancylostoma caninum*, *Dipylidium caninum* |

Since the vast majority of the species have heterogenous patterns of transmission, low values of prevalence also suggest that individual bobcats do not encounter infective stages of these parasites very frequently. Species considered in this category include *A. americana*, *A. mustelae*, *A. caninum*, *D. la- tum*, *D. mansonioides*, *M. barbatis*, *M. ingens*, *P. kelliotti*, *T. pseudolactoilis*, *T. pisiformis*, and *T. leonina*. All of these species are epizootic and most appeared with relatively low prevalence, which is considered a characteristic of generalist parasites that are able to infect a large number of individuals of different species [19]. Several of these parasites have a specific requirement to complete their life cycles and transmission to the definitive host [1, 8]. As a consequence, infective stages of parasites using intermediate and paratenic hosts would be consumed sporadically, resulting in infrequent reinfections that result in parasites showing low prevalence. For example, species of *Alaria* and *Dipylidium* must have a free-living life stage as well as aquatic intermediate hosts, which include planorbid snails and copepods, respectively.

No significant correlation was found between infection and intensity of any given parasite with age or sex. The only exception was infections caused by species of *Dipylidium*, which were more common in females than in males. This was opposite to the expected typical pattern of carnivores, with a sex bias toward males. Males generally have a higher parasite load than females in experimental situations and a slightly significant male bias has been documented in field studies [60]. We do not have a satisfactory explanation for this trend, and further testing would enable researchers to test if these patterns persist, and if temporal dietary fluctuations of females may explain this pattern. Furthermore, *A. caninum* was found more commonly in juveniles than in adults. This is consistent with previous findings, and it is a direct consequence of the host feline developing a stronger acquired immune system after initial infections [22, 25, 30]. Species of *Dipylidium* were found more commonly in trapped animals than in roadkills. This difference may be a factor of the restricted trapping area and small-scale habitat differences than as a result of the collection method; previous studies in southern Illinois found bobcat habitat was commonly intersected with roads [42, 43].

Rarefaction curves show that although a larger accumulation of helminth species is still expected, those species that may be considered common or specialists to bobcats were recovered as a result of our efforts. Bobcat-dwelling infracommunities tend to accumulate a large number of generalist species; however, there are five species that appear to be common to the bobcat, including *A. marcianae*, *T. rileyi*, and *T. cati*. The high variability and low concentration of dominant helminth species, indicated by the low Simpson's index, is consistent with previous studies and indicative of a parasite community dominated by a few species (*Taenia rileyi* and *A. marcianae*). Simpson's diversity index in bobcats from Illinois (0.13) is considerably similar to the value obtained for communities in Texas (0.1), even though the average species richness was lower for communities in Illinois relative to those in Texas, (2.4 vs. 7.4, respectively). Stone and Pence (1978) suggested that the differences in helminth communities...
likely reflect basic differences in specific collection localities, as well as regional differences. While different geographic areas may display different communities, it is localized home ranges that influence these infections.

The parasite community of bobcats in Illinois is relatively rich, with at least 18 species present. The comparison of bobcat parasite communities resulted in relatively low taxonomic similarity among component communities according to the low values of Jaccard’s index. This most likely can be attributed to relatively few shared parasites between the groups, since most of the parasite species occur in low prevalence in a single locality. Geographic distance has been shown to be an important determinant of the likelihood of presence of parasite species [48]. Bobcats, especially given their adaptability, reflect this variation. However, there is likely variation between localities that reflect the diverse habitats and prey species utilized by bobcats. Given the nidality of transmission of infectious diseases, Stone and Pence [67] suggested that differences among the composition of communities infecting individual bobcats were likely the result of the localized levels prevailing on the home range of an individual bobcat, rather than a regional effect. This may result in moderate or low similarity at the level of the component community detected in the dendogram (Figure 3).

The dendogram appears to show two clusters that reflect a putative meaningful regional similarity. This is seen in the clustering of communities from Minnesota and Nebraska (Semiarid Plains), and the Southeastern Temperate Plains (Georgia, Illinois, North and South Carolina, and West Virginia). The pattern appears to be challenged by the clustering of communities from Minnesota, Virginia, and New England that belong to three different geographic regions [3]. In this respect, a revision of the specimens used to make the identification appears to be necessary; however, at the present time it was not possible to locate the specimens from these three studies to complete the reexamination.

Areas in the US are experiencing a change in mammalian carnivore dynamics. Some areas: the Southeastern Temperate Plains, the Southeast Coastal Plains, and the Ozark/Ouachita/Appalachian Forest, have been highlighted as optimal for the potential recolonization of cougars (Puma concolor), which is congruent with the increase in cougar confirmed records in the region [32–34]. If cougars do indeed recolonize the Midwest, cougars will serve as an additional compatible definitive host for a large number of parasites, including the feline specialist T. cati. As cougars move east, they will be exposed to the parasites already present in the area, and will also have the potential of dispersing species from their native ranges [6, 27].

Although our study offers a baseline on the helminths present in bobcats in Illinois, more precision is necessary relative to the role of the season in the variations in helminth fauna, if any. This could be influenced by temperature changes, ability for larval development in the environment, and potential seasonal dietary shifts and how that may impact transmission from intermediate hosts [56]. These same factors could be used spatially as environmental variables to compare parasite presence based on specific locality. Correlations between species presence and environmental factors such as precipitation, land cover, or human density could provide further insight into localized infection areas. Geographic Information System (GIS) and related technologies are increasingly used to analyze the geography of disease, specifically the relationships between pathological factors and their geographical environments [14].

Very few of the helminths can be considered specific to felines, including T. rileyi and T. cati. Most of the species are generalists able to infect other carnivores; therefore, bobcats serve as competent reservoirs of agents of epizootic disease that may affect domestic carnivores. In the present study, three of the five species of helminths able to induce zoonotic disease were recorded; these species include P. kellicotti, D. mansonoides, and

Figure 2. Rarefaction curves of parasite species recovery per infracommunity in bobcats collected during 2003–2012. The Mau τ index was employed to calculate the observed species and the confidence intervals per node (infracommunity).
Toxocara cati. In addition, bobcats in Illinois are infected with another agent of zoonotic disease, D. latum.

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