Invited Review

Beyond the raccoon roundworm: The natural history of non-raccoon Baylisascaris species in the New World

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A total of 10 species of Baylisascaris, a genus of ascaridoid nematodes, occur worldwide and 6 of them occur in the New World. Most of the Baylisascaris species have a similar life cycle with carnivorous mammals or marsupials serving as definitive hosts and a smaller prey host serving as paratenic (or intermediate) hosts. However, one species in rodents is unique in that it only has one host. Considerable research has been conducted on B. procyonis, the raccoon roundworm, as it is a well-known cause of severe to fatal neurologic disease in humans and many wildlife species. However, other Baylisascaris species could cause larva migrans but research on them is limited in comparison. In addition to concerns related to the potential impacts of larva migrans on potential paratenic hosts, there are many questions about the geographic ranges, definitive and paratenic host diversity, and general ecology of these non-raccoon Baylisascaris species. Here, we provide a comprehensive review of the current knowledge of New World Baylisascaris species, including B. columnaris of skunks, B. transfuga and B. venezuelensis of bears, B. laevis of sciurids, B. devosi of gulonids, B. melis of badgers, and B. potosis of kinkajou. Discussed are what is known regarding the morphology, host range, geographic distribution, ecoepidemiology, infection dynamics in definitive and paratenic hosts, treatment, and control of these under-studied species. Also, we discuss the currently used molecular tools used to investigate this group of parasites. Because of morphologic similarities among larval stages of sympatric Baylisascaris species, these molecular tools should provide critical insight into these poorly-understood areas, especially paratenic and definitive host diversity and the possible risk these parasites pose to the health to the former group. This, paired with traditional experimental infections, morphological analysis, and field surveys will lead to a greater understanding of this interesting and important nematode genus.

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1. Genus Baylisascaris

1.1. History and relationships within Ascarididae

Baylisascaris is a genus within the family Ascarididae, comprising mostly heteroxenous nematodes with carnivorous definitive hosts. Baylisascaris procyonis, the raccoon roundworm, is by far the most well-known and extensively studied member of the genus, primarily because of its association with severe neurologic disease in humans and numerous species of animals. As a consequence, many other Baylisascaris species are relatively poorly studied compared to B. procyonis. Here, we review the life history and current knowledge of non-raccoon Baylisascaris spp. in the Americas.

The genus Baylisascaris was officially described in 1968 and was named in honor of parasitologist H. A. Baylis of the British Museum of Natural History (Sprent, 1968). This genus united some previous members of Ascaris and Toxascaris and was mainly differentiated from other ascarid genera based on the presence of pericloacal rough patches and subventral postcloacal papillae (versus the absence of subdorsal postcloacal papillae as in Toxascaris) (Sprent, 1968). Former members of Ascaris reassigned into Baylisascaris include B. devosi, B. columnaris, B. procyonis, and B. laevis, while B. transfigula and B. melis were formerly within the genus Toxascaris. While Baylisascaris and Toxascaris share biological similarities and are often discussed together in the context of zoonotic ascarids, they are in different subfamilies and are well-separated within Ascarididae (Nadler and Hudspeth, 2000). Molecular phylogenetic analyses of several genetic targets also support the separation of Baylisascaris from other ascarid genera (Zhu et al., 1998; Nadler and Hudspeth, 2000; Fransson et al., 2013; Tokiwa et al., 2014).

1.2. Life cycle characteristics

With the exception of B. laevis, all members of Baylisascaris utilize a carnivore definitive host. Most of these carnivore-infecting species also utilize a wide range of natural paratenic hosts (although there are some data to suggest these hosts are intermediate hosts, see below). Adult nematodes develop in the small intestinal lumen of the definitive host where they feed on host digesta. Females are remarkably fecund and can release >100,000 eggs/worm/day (primarily based on data from B. procyonis), which are shed in the feces (Snyder and Fitzgerald, 1987). Over a variable period of time (10–14 days under ideal conditions), the zygote within the egg develops into an infective-stage larva that may infect either definitive hosts in a direct cycle, or paratenic hosts in an indirect cycle (Fig. 1). However, it is likely that definitive hosts acquire some immunity to infection via the direct route with age; experimental infections show that egg inoculation can generally only establish infection in young definitive hosts (Kazacos, 1983; Berry, 1985). Like other ascarids, Baylisascaris spp. eggs are covered in an adhesive proteinaceous coat that confers a high degree of resilience to desiccation, freezing, heat (up to 62 °C), and disinfectants, and may remain viable in the environment for years (Shafir et al., 2007; Kazacos, 2016).

When ingested by paratenic hosts, larvae hatch from eggs in the small intestine, penetrate the intestinal wall, and undergo tissue migration after entering circulation. The pattern of migration and the resulting larva migrans syndromes vary among Baylisascaris spp. and host species. In B. procyonis, three larva migrans syndromes are well-described: visceral larva migrans (VLM), ocular larva migrans (OLM), and neural larva migrans (NLM), the latter of which can cause severe neurologic disease with permanent sequelae and death (Kazacos, 2016). Migrating larvae often become encapsulated in paratenic host tissues and are infective to definitive hosts upon predation. Once ingested by the definitive host, it is presumed L3 larvae mature in the mucosa of the small intestine, returning to the lumen at the L4 stage and molting into adults, as has been shown with Toxascaris, although it is not clear whether further somatic and/or tracheal migration takes place within all definitive hosts (Sprent, 1954).

It is possible that the route of infection determines whether further migration occurs within the definitive host. Assuming that the stage within the egg is L2, after egg inoculation, migration out of the gastrointestinal tract may be required for advancement to L3. However, if infection occurs via the ingestion of L3 larvae in host tissues, somatic migration may not be necessary; maturation may occur solely in the intestinal wall and lumen without migration, as has been shown with Toxascaris (Sprent, 1954). Phylogenetically, Baylisascaris forms a sister clade with other Ascarididae (e.g., Toxascaris, Ascaris, Parascaris) although within this group,
Toxascaris is the only member not known to undergo larva migration during development (Li et al., 2012). Experimental data on differential infection dynamics based on exposure route for Baylisascaris are lacking (Sprent, 1954; Nadler and Hudsphet, 2000). However, a skunk orally inoculated with B. columnaris eggs had an L3 recovered from skeletal muscle whereas this was not observed in other skunks in the same trial that had been inoculated with infected mouse carcasses (Berry, 1985). Migrating Baylisascaris sp. larvae have been recovered from skeletal muscle of naturally-infected wild definitive hosts but route of infection is unknown (Hoberg et al., 1990).

Debate exists as to whether hosts in which larvae migrate should be referred to as paratenic or intermediate hosts, which would be dictated by the presence of either a L2 or L3 larvae within the egg. In developmental observations of Baylisascaris tasma-niensis, changes in the oral region and a duplication of the cuticular sheath were observed at six days post infection in laboratory mice, which is evidence that a second molt occurs during larva migrans (i.e. L2 to L3) (Sprent et al., 1973). Additionally, only one molt was described within the egg after differentiation of the embryo of B. tasmaniensis, so it is likely that larvae within fully developed eggs of this species represent the L2 stage and not L3 (Sprent et al., 1973). Similar observations were also reported in Baylisascaris laevis (Babero, 1960a,b). However, when hatched from eggs for in vitro culture, B. procyonis larvae were partially encased in a thin cuticle and did not molt further; it is not clear whether this cuticle represents a product of the first molt or a second molt within the egg (Boyce et al., 1988). Descriptions of Toxocara canis larval development suggest this thin cuticle is an artifact of the first stage cuticle, and could be misinterpreted as a second in ovo molt (Schacher, 1957). It is also possible that certain Baylisascaris species molt twice within the egg while others molt only once.

Whether or not the second molt occurs within the host or in the egg of Baylisascaris spp. and related ascaridoids, migrating Baylisascaris spp. larvae grow extensively while migrating through host tissues (depending on species, from ~200 μm to ~1800 μm over the course of weeks). It is possible this growth confers some advantage to larvae as infection efficiency in mature definitive hosts is remarkably higher when inoculated with larvae (i.e. infected carcasses) versus eggs and prepatent periods are shorter (Kazacos, 1983; Miyashita, 1993; Berry, 1985). This suggests that some larval development during somatic migration in non-definitive hosts.

1.3. Diagnostic features

Grossly, Baylisascaris spp. adults appear very typical of large ascarids (long cylindrical body, off-white to brown in color, three prominent lips, tail tapered to a point), with the females larger than males. Sprent (1968) described the main diagnostic features of the genus Baylisascaris as being:

- Males have a roughened area anterior and posterior of the cloaca, unlike Ascaris or Toxascaris; post-cloacal papillae arranged differently from Toxascaris
- Includes all characteristics within Ascarididae.
- Cervical alae present, either salient or reduced.
- Dorsal and sub-ventral labial papillae present in doublets.
- Excretory cell U-shaped, nucleus within or behind commissural region.
Table 1
Overview of Baylisascaris species endemic to the New World, including *B. procyonis* for comparison.

| Species                  | B. procyonis | B. columnaris | B. devosi | B. laevis | B. melis | B. potosis | B. transfuga | B. venezuelensis |
|--------------------------|--------------|---------------|-----------|-----------|----------|------------|--------------|------------------|
| Authority                | Stefancki and Zarnowski, 1951 | Leidy, 1851 | Sprent, 1952a,b | Leidy, 1851 | Gedeost, 1920 | Tokiwa et al., 2014 | Rudolfi, 1819 | Perez, Garcia & Gau, 2015 |
| Historical synonyms      | Ascaris procyonis, Ascaris columnaris “raccoon ascariid” | Ascaris alienata, Ascaris columnaris “skunk ascariid” | Ascaris guanion, Ascaris mustelatum, Ascaris devosi | Ascaris laevis, Ascaris tarbogan | Belascaris melis, Toxascaris melis, Ascaris columnaris “badger ascariid” | N/A | Ascaris transfuga, Toxascaris transfuga, Baylisascaris multipapillata Bears (Ursus, Melursus) | N/A |
| Primary DH (genus or species) | Raccoons (Procyon lotor); Domestic dogs (Canis familiaris) | Skunks (Mephitis, Spilogale) | Fishers, Martens, Wolverines (Martes, Gulo) | Marmots, ground squirrels (Marmota, Spermophilus) | Badgers (Meles, Taxidea) | Kinkajou (Potos flavus) | Spectacled bear (Tremarctos ornatus) |  |
| Adult length (d; f)      | 46 - 119; 55 - 337 | 43 - 110; 72 - 266 | 57 - 123; 105 - 285 | 37 - 108; 32 - 212 | 120 - 127; 22 - 260 | 117 - 123; 214 - 221 | 216 - 224 | 63 - 120; 102 - 240 |
| Adult midbody width (d; f) | 1.0 - 2.0; 1.5 - 2.5 | 1.3 - 2.5 | 1.3 - 2.5 | 1.0 - 2.3; 1.7 - 3.2 | up to 3; up to 5 | 1.5 - 1.75; 2.51 - 2.9 | 1.2 - 1.9; 1.6 - 4.5 | 102; 250 |
| Esophageal length (d; f)  | 2.19 - 6.46; 2.41 - 7.53 | 2.69 - 5.21; 3.76 - 7.81 | 4.15 - 4.50; 4.00 - 8.43 | 2.73 - 5.64; 1.84 - 6.59 | 4.50; 4.00 | 5.9; 7.25 | 4.10 - 4.56 | ND |
| Spicules                  | 0.49 - 0.71; 0.25% | 0.33 - 0.76; 25% | 0.39 - 0.54; 33% | 0.24 - 0.81; 34% | 0.80 - 0.90 | 0.61 - 0.77 | 0.80 - 0.93; 0.9 | 0.4 | 0.9 |
| Vulvar position           | 43 - 67 | 36 - 53 | 30 - 40 | 41 - 61 | up to 63 | 44 - 52 | 46 - 70 | ND |
| Number of pre-anal papillae | 80 x 60 μm; Sprent 1968, Berry 1985, Kazacos 2016 | 73 x 63 μm; Sprent 1968, Berry 1985 | Inconspicuous 77 x 61 μm; Sprent 1952a,b, Sprent 1953b, Sprent 1968 | Inconspicuous 75 x 62 μm; Babero 1960a, Sprent 1968, Berry 1985 | Prominent 87 x 75 μm; Gedeost 1920, Hartwick 1958, Sprent 1968 | Prominent 83 x 73 μm; Tokiwa et al., 2014 | Prominent 90 x 75 μm; Rudolfi 1819, Sprent 1968, Testini et al., 2011, Moudgil et al., 2014 | Prominent ND |
| Cervical alae            | Inconspicuous 80 x 60 μm; Sprent 1968, Berry 1985, Kazacos 2016 | Inconspicuous 73 x 63 μm; Sprent 1968, Berry 1985 | Inconspicuous 77 x 61 μm; Sprent 1952a,b, Sprent 1953b, Sprent 1968 | Inconspicuous 75 x 62 μm; Babero 1960a, Sprent 1968, Berry 1985 | Prominent 87 x 75 μm; Gedeost 1920, Hartwick 1958, Sprent 1968 | Prominent 83 x 73 μm; Tokiwa et al., 2014 | Prominent 90 x 75 μm; Rudolfi 1819, Sprent 1968, Testini et al., 2011, Moudgil et al., 2014 | Prominent ND |
| Average egg size         | 70 - 100; 250 μm; Sprent 1968, Berry 1985, Kazacos 2016 | 70 - 100; 250 μm; Sprent 1968, Berry 1985 | 70 - 100; 250 μm; Sprent 1968, Berry 1985 | 70 - 100; 250 μm; Sprent 1968, Berry 1985 | 70 - 100; 250 μm; Sprent 1968, Berry 1985 | 70 - 100; 250 μm; Sprent 1968, Berry 1985 | 70 - 100; 250 μm; Sprent 1968, Berry 1985 | 70 - 100; 250 μm; Sprent 1968, Berry 1985 |

ND = not determined.

a Measurements in mm unless otherwise specified.
b Percentage of body length from anterior end.
c Inconspicuous = only visible in transverse section.
d Measurement represents examination of single specimen.

- Relatively short, stout spicules less than 1.0 mm in most members.
- Male tail papillae are segregated into pre- and post-cloacal groups with varying numbers depending on species. The post-cloacal group comprises a pairs in doublets, a single pair of closely associated singlet papillae, and one phasmod.
- Eggs ovoid to round and finely pitted.

Among the majority of *Baylisascaris* spp., considerable overlap occurs in the morphology of eggs, larva, and many adult characteristics (Table 1). However, there are some morphologic characteristics that can be used to distinguish the various species. For example, *B. transfuga* and *B. melis* are generally thicker and more stout in overall appearance than other *Baylisascaris* spp., have larger spicules, and have cervical alae that are grossly visible (Table 1) (Sprent, 1968). Distinguishing among *B. procyonis*, *B. columnaris*, *B. devosi*, *B. potosis*, and *B. laevis* on morphology alone is difficult or impossible, although *B. laevis* is generally smaller than the other species and although there is overlap, there are some differences in spicule length, number of pre-anal papillae or vulval position (Table 1). Most species are identified based on definitive host alone. However, because some *Baylisascaris* spp. can cross-infect various definitive host species, species identification should not be based on definitive host species alone (Berry, 1985; Sprent, 1952a; Kazacos, 2016). Molecular tools for species identification are readily available and should be used when possible to confirm identifications if needed (see Section 3).

2. New World species of *Baylisascaris*

2.1. *Baylisascaris columnaris*

Originally identified as *Ascaris alienata* by Leidy (1851), the species was renamed *Ascaris columnaris* in 1856, and then reasigned to *Baylisascaris* by Sprent in 1968 (Leidy, 1851; Sprent, 1968). Morphologically, *B. columnaris* is highly similar to *B. procyonis*, with only subtle distinguishing features, including the structure of cervical support in the cuticle (a wide arch in *B. procyonis* compared with a narrow A-shape in *B. columnaris*), shape of denticles (equilateral triangles versus elongated triangles), male tail terminal shape (spike versus knob), and the number of preanal papillae (average of 40 versus 43, although there is considerable overlap in range) (Franssen et al., 2013; Sprent, 1968). However, it is likely that enough natural variability occurs between the two species such that identification based only on morphological characteristics is inadequate and molecular identification is ideal (Berry, 1985).

Skunks are the definitive host for *B. columnaris*. Infection has primarily been detected in the striped skunk (*Mephitis mephitis*), the most broadly-distributed and studied skunk species in North America, but infections have also been detected in Eastern spotted skunks (*Spilogale putorius*) (Table 2). Symptomatic Western spotted skunks (*Spilogale gracilis*) and hog-nosed skunks (*Conepatus sp.*) may be potential hosts as well but testing has been limited. Ascariids identified as *B. columnaris* have been reported in American badgers (*Taxidea taxus*); however, it is likely that these are actually *B. melis* or *B. devosi* (Table 2). Further surveys that utilize molecular
parasite species identification tools will be useful in elucidating the full definitive host range of *B. columnaris*.

### 2.1.1. Distribution and ecology

Contemporary surveys on *B. columnaris* are generally lacking; more surveillance is needed to accurately characterize the distribution and eco-epidemiology of this parasite because most recent reports are from captive pet skunks (d’Ovidio et al., 2016). From published reports, *B. columnaris* appears to be well-established in the northeast, upper Midwest, and prairie regions of the United States and Canada, apparently uncommon in arid regions of the west and southwest (Table 2). In southern Ontario, Canada, prevalence in *M. mephitis* was significantly lower in spring months compared to late summer or early fall (Berry, 1985), similar to the general seasonal patterns observed for *B. procyonis* in northern climates (Kidder et al., 1989). Similar seasonal variation in prevalence and intensity have been observed for other gastrointestinal helminths of skunks, such as *Physaloptera maxillaris* (Cawthorn and Anderson, 1976). It is likely that the resource-limiting nature of skunk torpor/overwinter fasting causes the loss or developmental arrest of helminths, potentially including *B. columnaris*, but more research is needed to investigate this phenomenon (Dragoo, 2009).

### 2.1.2. Natural infections in definitive hosts

Similarly to *B. procyonis* in raccoons, *B. columnaris* infection is generally not associated with morbidity or mortality in wild skunk definitive hosts. However, peritonitis or intestinal perforation associated with high worm burdens in captive skunks have been documented (Goodey and Cameron, 1923; Nettles et al., 1978). Goodey and Cameron (1923) noted that skunks from a United Kingdom fur farm exhibited poor body condition, failure to thrive, and inferior coat quality possibly associated with high-intensity *B. columnaris* infection, and possibly resulting in economic losses. Few recent studies have investigated the occurrence of *B. columnaris* among farmed skunks or its economic impacts, although infection control should be straightforward with appropriate enclosure cleaning and regular administration of anthelmintics, most of which are highly efficacious against intestinal stages of the related parasite *B. procyonis* (Bauer and Gey, 1995). Given these data, it is possible that wild skunks with high worm burdens may develop disease.

In Europe, 15 of 60 (25%) pet striped skunks primarily from Germany and Italy were positive and additional infections have been reported from the Netherlands and Poland; all were genetically-confirmed as *B. columnaris* (Franssen et al., 2013; d’Ovidio et al., 2016; Janczak et al., 2016). Worm burdens were not determined, but in one study, eggs per gram of feces (EPG) ranged from 150 to 14,500 (mean of 4713 EPG) (d’Ovidio et al., 2016). This is relatively low compared to natural *B. procyonis* infections, which may average 26,000 EPG (Kazacos, 2016). Importantly, many of these infected pet skunks were housed near or with other pet species (e.g. dogs, guinea pigs, parrots), and none of the skunks had ever received anthelmintic treatment (d’Ovidio et al., 2016). Although there are no published reports, *B. columnaris* infections have been diagnosed in pet skunks from the United States (Yabsley, unpublished data). Given the popularity of skunks as pets and the potential for larva migration in various hosts, education of pet owners is needed to reduce the risk of transmission.

### 2.1.3. Experimental infections in definitive hosts

Experimental infections of *B. columnaris* in skunks have provided data on the fate of larvae within the definitive host and the development of patency. Berry (1985) experimentally infected striped skunks by feeding them mouse carcasses containing unknown numbers of L3 larvae. Two juvenile female skunks became patent ~48 days post-inoculation (DPI) and one mature male skunk became patent at 93 DPI. Another route of exposure investigated was inoculation with larvated eggs; inoculation of an unspecified number of embryonated eggs resulted in intestinal infections in three juvenile skunks. The youngest individual (38 days old) was sacrificed at 10 DPI and one L3 larva was recovered from skeletal muscle, suggesting that at least some larvae undergo early somatic migration in the definitive host following egg inoculation. This individual also had a small number of L3 and L4 larvae within the lumen of intestine. Numerous L3 and L4 larvae were observed in the lumen of the small intestine in another juvenile “young of the year” skunk sacrificed at 19 DPI; additional larval larvae were recovered via digestion of the walls of the anterior and posterior small intestine. The remaining juvenile animal was sacrificed at 139 DPI, and although immature adults were found in the intestine at necropsy, eggs were never detected in the feces. The limited number of experimental infection trials in skunks and the low sample sizes makes determining the average onset of patency difficult, and host age and route of infection may be important factors not fully investigated.

To investigate susceptibility of raccoons to *B. columnaris*, two juvenile raccoons were inoculated with unreported numbers of either L3 larvae (in mouse tissue) or embryonated eggs (Berry, 1985).
1985). The raccoon inoculated with larvae became infected as L4 larvae were present in the small intestine of the raccoon upon necropsy; however, infection was not allowed to proceed so it is unknown if the raccoon would have become patent. Thus it is unknown if raccoons can serve as alternative definitive host for *B. columnaris*. The single raccoon inoculated with embryonated *B. columnaris* eggs did not develop an intestinal infection (Berry, 1985).

2.1.4. Natural infections in non-definitive hosts

There are no reports of naturally-acquired *B. columnaris* larva migrants in wild or captive paratenic hosts. There have been suspected cases in captive animals that were linked to co-housing with infected skunks. For example, an outbreak involving a white-headed marmoset (*Callithrix geoffroyi*) and two species of tamarins (*Saguinus nigricollis*, *Saguinus midas*) in a zoological park in Texas was likely due to a skunk (of unknown infection status) housed in the enclosure. These primates developed signs of NLM, were treated unsuccessfully with fenbendazole, and were subsequently euthanized (Huntress and Spraker, 1985). Infection with *B. columnaris* in a captive emu (*Dromaius novaehollandiae*) in Indiana with fatal NLM was suspected based on the history of a skunk (also of unknown infection status) being previously held in the enclosure (Kazacos et al., 1982). However, species identification was not confirmed in the emu case. Raccoons are also reportedly common in the area where the emu was housed and *B. procyonis* is highly prevalent in Indiana (Kazacos et al., 1982).

The paratenic host range of *B. columnaris* is likely broad given its biological and phylogenetic similarity to *B. procyonis* and experimental host range. However, molecular techniques will be required in future case studies or surveys to investigate possible natural paratenic hosts. Additionally, *B. columnaris* could be a zoonotic parasite, given its similarities to *B. procyonis* and case reports in primates. It is possible that some presumed *B. procyonis* natural infections are actually *B. columnaris*, due to the extreme difficulty of species identification through adult/larval morphology, egg morphology (from environmental samples), or current serologic techniques which are cross-reactive among *Baylisascaris* spp. (Dangoudoubiyam et al., 2010; Berry, 1985). No human cases have been reported, and even if zoonotic, it is unlikely to represent as significant a public health threat as *B. procyonis*, as skunks are generally in lower densities in urban areas compared to raccoons (Gehrt, 2004). Therefore, potential human contact with skunks appears limited. Nonetheless, individuals with frequent contact with skunks and skunk feces (pet owners, wildlife rehabilitators, fur farmers, trappers, etc.) should take precautions against potential exposure to *B. columnaris*. Recently, antibodies to *Baylisascaris* (presumed to be mostly due to *B. procyonis* exposure, but could be due to other species) were detected in wildlife rehabilitators (Sapp et al., 2016a).

2.1.5. Experimental infections of non-definitive hosts

*B. columnaris* produces disease due to larva migrants in a variety of experimentally-infected paratenic host species, particularly rodents and lagomorphs. Compared to *B. procyonis*, *B. columnaris* generally causes less mortality in experimentally-infected rodents due to slower and more limited NLM. Independent experiments by Sprent (1952b) and Tiner (1953a) demonstrated that neurological signs were generally noted between 17 and 25 days in laboratory mice inoculated with an unspecified number of eggs, compared to 7–10 days for *B. procyonis*. However, dose is likely important in the rate of disease development; as has been shown with *B. procyonis* (Tiner, 1953a; Sheppard and Kazacos, 1997; Sapp et al., 2016b). Domestic rabbits (*Oryctolagus cuniculus*) inoculated with 100,000 eggs, considered a very high dose, rapidly developed severe neurologic disease involving seizures, epistaxis, ataxia, and dyspnea, with onset between 4 and 10 DPI (Church et al., 1975).

This generally delayed onset of neurological disease compared to *B. procyonis* is most likely due to the relatively slower growth of L3s within paratenic hosts. In a 20 day trial of experimental infection of laboratory mice, *B. columnaris* larvae grew to approximately 1000 μm in length by the end of the trial, compared to *B. procyonis* that achieved this size by day 10 and reached an average maximal length of 1200 μm by day 20 (Tiner, 1953b). Experimental trials in laboratory mice suggest that neurological disease does not become readily apparent until larvae within the brain have reached a length of ~1000 μm (Tiner, 1953b) in laboratory mice, *B. procyonis* reaches an average length of ~1000 μm in ~8–10 days post infection, after which survival of infected hosts fell dramatically, whereas *B. columnaris* took ~16 days to reach 1000 μm in length, after which time some mortality occurred (Tiner, 1953b). In some cases, mice inoculated with *B. columnaris* eggs were able to recover from clinical disease or survived despite the presence of larvae in the brain (Tiner, 1953b). Similarly, *B. procyonis* larvae have been detected in wild-caught, presumably normally-acting, *Peromyscus leucopus* further suggesting that some rodents can survive infections of the brain (Page et al., 2001; Sapp and Yabsley unpublished data). The observed differences larval growth between *B. columnaris* and *B. procyonis* may also reflect differences in paratenic host species adaptation. In experimentally-infected meadow voles (*Microtus pennsylvanicus*), *B. columnaris* larvae achieved a greater average length (1570 μm) than *B. procyonis* (1060 μm) by 10 DPI (Berry, 1985). The study was terminated at 10 DPI for larval morphological analysis, so other infection dynamics were not assessed. The impact of larval size on disease severity could also explain why cerebral infection with other smaller larval ascarids such as *Toxocara canis* (that achieves a maximal length of about 500 μm in rodent brains) produces overt disease much less frequently than *B. columnaris* or *B. procyonis*, although host factors, including brain size, likely play a role in the rate and severity of neurologic disease (Tiner, 1953a; Sprent, 1955).

A large proportion of larvae in inoculated paratenic host species become encapsulated within the intestinal wall and mesentery within 1–4 DPI (Berry, 1985). Encapsulated larvae were also abundant in the lungs, heart, kidneys, and liver shortly after inoculation in laboratory mice and in a groundhog (*Marmota monax*), presumably due to liver-lung migration (Sprent, 1952b; Berry, 1985). Numerous larvae migrated within skeletal muscle and became encapsulated after 10 DPI in inoculated *Microtus pennsylvanicus* (Berry, 1985). In inoculated experimentally-infected rabbits, extensive larval granulomas with eosinophilic infiltration were observed in the lungs, liver, brain, eyes, kidneys, heart, and gastrointestinal tissues (Church et al., 1975).

Similar to other *Baylisascaris* species, *B. columnaris* larvae within tissues are resistant to freezing. Encapsulated larvae in tissues remained viable and recovered substantial motility after a periods of freezing ranging from 8 to 18 weeks at ~20 °C, which was superior to freeze-susceptible *B. transfuga* and *T. canis* larvae in the same experiment (Sprent, 1953a). However, experimental trials to confirm infectiousness of previously-frozen *Baylisascaris* larvae have not been conducted.

2.2. *Baylisascaris* spp. of bears

2.2.1. *Baylisascaris* transfuga

*Baylisascaris transfuga* was originally described by Rudolphi (1819) as *Ascaris transfuga* and re-described as a *Toxascaris* species in 1922 (Baylis and Daubney, 1922). Ultimately Sprent (1968) formally described the *Baylisascaris* genus and designated *Baylisascaris transfuga* as the type species for this genus. Morphological
characteristics and/or molecular techniques can be used to distinguish this species from other Baylisascaris spp. (Table 3). Baylisascaris transfuga adults can be morphologically distinguished from other species by their spicule length (estimated between 0.80 and 0.92 mm), having between 46 and 70 precloacal papillae, rounded posterior margin of the percloacal area, salient alae, denticles in equilateral triangles, and a saddle shape of the median lobe of the lip (Testini et al., 2011; Sprent, 1968; Baylis and Daubney, 1922). Eggs of *B. transfuga* are morphologically similar to other *Baylisascaris* spp. eggs and are largely considered indistinguishable (Sprent, 1968; Kazacos and Turek, 1983; Testini et al., 2011) (Table 1).

Of interest is that morphometrics of parasites reported as *B. transfuga* from numerous hosts across several continents show variation (Table 3). Measurements have been made on specimens from black bears (*Ursus americanus*) in Canada, multiple subspecies of brown bear (*Ursus arctos*) from across sites Eurasian and North American sites, multiple captive polar bears (*Ursus maritimus*) in various sites, sloth bears in India (*Melursus ursinus*), and a captive sun bear (*Helarctos malayanus*) (Canavan, 1929; Baylis and Daubney, 1922; Sprent, 1968; Testini et al., 2011; Moudgil et al., 2014). Sprent (1968) reported a maximal length of 120 mm for males and 240 mm for females; other studies have found smaller measurements on *B. transfuga* (*Canavan, 1929; Baylis and Daubney, 1922; Testini et al., 2011*), having between 46 and 70 precloacal papillae, rounded egg dimensions are also variable and have been reported as low as ~57 µm wide with up to ~94 µm long, although fertilization or embryo formation status may influence this morphology (Table 3).

These morphologic differences in a limited number of parasites examined across a wide geographic and host range suggest that these "*B. transfuga*" may represent several distinct species. Currently there are two distinct *Baylisascaris* species apart from *B. transfuga* reported within Ursidae, *B. Schroederi* of giant pandas (*Ailuropoda melanoleuca*), and the recently-described *B. venezuelensis* from spectacled bears (*Tremarctos ornatus*). Further support is provided by preliminary molecular data on *B. transfuga* samples from Alberta, Canada and West Virginia that suggest these parasites are genetically distinct across locations (L. Camp, pers. comm.). Careful morphologic analysis combined with molecular characterization of *B. transfuga* samples from a diverse geographic and host range is needed to address parasite diversity in the Ursidae.

### 2.2.1.2. Non-definitive hosts

The prevalence of *B. transfuga* in bears varies widely among studies (Table 4). In North America, the majority of studies have been conducted on black bears but natural infections have been reported in brown bears and in captive polar bears. Prevalence of *B. transfuga* in black bears appears to be highest in Alberta, Canada and the Great lakes regions of the USA. In brown bears, prevalence of infection with *B. transfuga* was higher in the Wyoming and Montana, USA compared to Alaska and Canada. A few studies have attempted to investigate seasonal trends in prevalence with some conflicting results. One, based on fecal floatation, detected a higher prevalence in spring compared to the fall in black bears in Quebec, Canada (Frechette and Rau, 1978). Another, based on collection of nematodes at necropsy in black bears and grizzly bears in western Canada observed the opposite seasonal trend with peaks in the fall but this study was (Frechette and Rau, 1978; Catalano et al., 2015). However, the seasonal association with prevalence in black bears was weakly significant (p = 0.04) and sample sizes were relatively small (n = 40); sample size for grizzly bears was too small for statistical analysis. Finally, Rausch (1954) and Rogers (1975) found that bears were shedding eggs in the spring soon after torpor and found evidence of egg shedding just prior to denning, so it remains unclear whether or not infections are cleared during winter torpor. Additional studies are needed with greater sample sizes and age class representation to accurately assess the seasonal ecology of *B. transfuga* in bears.

In the bear host, *B. transfuga* infections do not typically cause clinical disease, but heavy infections have been reported causing clinical disease or death. Reports of disease in the natural bear host include peritonitis in a brown bear in Europe and suggestions of enteric impactions (Mozgovoi, 1953; Szczepaniak et al., 2012). Subclinical effects including reduced host condition have also been reported in infected bears (Fu et al., 2011). Despite a reduced migratory capacity relative to other *Baylisascaris* spp., experimental infections in laboratory mice, Mongolian jirds (*Meriones unguiculatus*), guinea pigs, rabbits, and chickens indicate that *B. transfuga* can occasionally cause VLM, OLM, and/or NLM; however, in most hosts, clinical disease was mild or not apparent (Sprent, 1952b, 1953a; Papini et al., 1993, 1999a; Papini and Casarosa, 1994; Sato et al., 2004; Matoff and Komandarev, 1965). There is substantial variation among hosts in disease severity. Laboratory mice developed only mild clinical disease with granulomas in the brain. Mongolian jirds developed severe clinical signs with malacia and lack of host immune reaction (Sato et al., 2004). Rabbits displayed a loss of appetite, dyspnea, and depression but no neurological signs (Papini et al., 1999a). No

| Host | Location | Male length (mm) | Female length (mm) | Egg width (µm) | Egg length (µm) | Source |
|------|----------|------------------|--------------------|----------------|----------------|--------|
| Multiple captive and wild species | Multiple | Up to 120 | Up to 240 | Up to 75.0 | Up to 90 | Sprent 1968 |
| Sloth bear (*Melursus ursinus*) | India | 64–94 | 138–183 | 47.0–75.2 | 65.8–94.0 | Moudgil et al., 2014 |
| Polar bear (*Ursus maritimus*) | Italy | 63–116 | 102–203 | NG | NG | Testini et al., 2011 |
| Multiple captive species | Pennsylvania (USA) | 81–142 | 108–166 | NG | NG | Papini and Casarosa 1994 |
| Multiple captive species | Louisiana (USA) | NG | 66.3–74.7 | 78.3–88.0 | Clark et al., 1969 |

NG – not given.
larvae were not noted in brains. Sprent (1953a) reported that larvae migrate into the intestinal wall and either encyst in the wall, penetrate the intestinal wall and enter the bloodstream through the lymphatics and systemic circulation. However, another study showed that larvae migrans, only one presumed report of natural infection.

Despite experimental studies suggesting a wide range of hosts may develop larva migrans, only one presumed report of natural infection of a paratenic host has been reported. Japanese macaques (Macaca fascicularis) housed near American black bears at a zoo in Japan developed fatal neurological disease; however, identification of the parasite was not definitive in this case and identification of the parasite was based only on histology (Sato et al., 2005). While there are no confirmed reports of larva migrans in humans following B. transfuga infection, experimental evidence with other species shows that given a sufficiently high infection, larva migrans may develop in people.

2.2.1.3. Treatment and control. Baylisascaris eggs present in the environment or in captive animal facilities are difficult to eliminate or kill. Similar to other Baylisascaris species, eggs of B. transfuga become infective after ~2 weeks and can remain infective for at least 15 months under artificial conditions (Papini et al., 1993). Eggs have reported to persist in the environment for up to five years, and infected bears can pass between 100 and 19,800 eggs per gram of feces so environments can become contaminated with large numbers of eggs quickly (Abdel-Rasoul and Fowler, 1979; Vercruysse et al., 1976). In captivity, the prevalence of B. transfuga is higher in certain species (U. maritimus, Melursus ursinus), although this could be sampling bias, the substrate (e.g. sand or soil) used in enclosures, or the housing of bears in groups (Schaul, 2006). Strict, routine quarantine and treatment of bears in captive settings can be an effective way to reduce shedding and prevent subsequent infections.

Treatment of bears infected with B. transfuga has only been done in captive situations. Numerous anthelmintics have been used to manage B. transfuga infections in captive bears; however, efficacy is variable and dose-dependent (Clark et al., 1969; Moudgil et al., 2014). Dichlorvos (19 mg/kg) rapidly (1–2 days post treatment) reduced fecal egg counts (FEC) to zero in many bear species; however, these animals became reinfected within months after treatment, emphasizing the need to clean the environment (Clark et al., 1969). Orally-administered fenbendazole (10 mg/kg) on three consecutive days was unable to reduce fecal egg counts to zero in a sloth bear, however, this infection was cleared with 15 mg/kg for three days followed by the original treatment (Moudgil et al., 2014). Mebendazole was used successfully to treat five polar bears infected with B. transfuga (Vercruysse et al., 1976). Macrolides, benzimidazoles, and tetrahydropyrimidines have all been used in North American zoos to treat bears but efficacy data were not provided (Schaul, 2009).

Due to concerns about larva migrans, it is important to determine if larvae could be killed prior to entering the CNS. Laboratory mice given one dose of 2 mg/kg ivermectin had fewer lesions and larvae recovered in visceral organs (at 14 DPI). Although ivermectin had similar activity against larvae at 3 DPI (88% reduction), it had greater activity against larvae at 14 DPI (75% reduction).

Table 4: Reports of Baylisascaris transfuga in free-ranging and captive bears in North America.

| Host Location                | No. infected/no. examined (%) | Method of detection | Source            |
|-----------------------------|------------------------------|---------------------|-------------------|
| Brown bear                  |                              |                     |                   |
| Northwest Territories, Canada Wild  3/96 (5) Fecal flotation Gau et al., 1999 |
| Wyoming and Montana, USA Wild  53/70 (76) Necropsy Worley et al., 1976 |
| Canada Wild  16/21 (76) Necropsy Choquette et al., 1969 |
| Alaska, USA Wild  0/28 (0) Fecal flotation Schaul, 2006 |
| British Columbia and Alberta, Canada Wild  7/13 (54) Necropsy Catalano et al., 2015 |
| Black bear                  |                              |                     |                   |
| Florida, USA Wild  5/22 (23) Necropsy Foster et al., 2004 |
| New York, USA Wild  17/55 (31) Necropsy King et al., 1960 |
| Minnesota and Michigan, USA Wild  5/9 (56) Necropsy Rogers, 1975 |
| Wisconsin, USA Wild  59/92 (64) Fecal flotation Manville, 1978. |
| Alberta, Canada Wild  25/29 (86) Necropsy Worley et al., 1976 |
| Quebec, Canada Wild  17/30 (58) Necropsy Dufy et al., 1994 |
| Quebec, Canada Wild  13/18 (29) Necropsy Dies, 1979 |
| Ontario, Canada Wild  42/22 (60) Necropsy Addison et al., 1978 |
| British Columbia and Alberta, Canada Wild  24/40 (60) Necropsy Catalano et al., 2015 |
| Northwest Territories, Canada Wild  12/27 (44) Fecal flotation Johnson et al., 2013 |
| Polar bear (U. maritimus) Massachusetts, USA Captive  18/28 (64) Necropsy McRostie et al., 2002 |
| U. arctos, U. maritimus California, USA Captive  2/3 (66) Necropsy Abdel-Rasoul and Fowler, 1979 |
| Various USA, various Captive  125/260 (48) Fecal flotation Schaul, 2006 |

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suggest that larvalcidal activity will vary depending on the age of infection, resulting in changes in susceptibility to the drugs during migration, and in encapsulation status. Pharmacokinetics of anthelmintics also influence treatment efficacy; for example, ivermectin does not cross the blood-brain barrier whereas levamisole appears to do so (Fox, 2006; Lin and Tsai, 2006). Given this differential susceptibility and drug efficacy, it seems the best option for treating B. transfuga larva migrans in paratenic hosts is to use multiple drug classes in order to maximize larvalcidal activity in brain, viscera, and skeletal muscle.

2.2.2. Baylisascaris venezuelensis

A new species, Baylisascaris venezuelensis, originating from a South American spectacled (Andean) bear (Tremarctos ornatus) in western Venezuela was recently described (Pérez Mata et al., 2016). A female spectacled bear in poor body condition was found dead and at necropsy, a large number of Baylisascaris were present in the gastrointestinal tract. Other gross pathologic findings included congestion and hemorrhagic foci in the lungs. The authors suggest that the high worm burden was the cause of mortality.

An adult male and female nematode were examined morphologically. Fewer post-oculcaal papillae (n = 44) were present compared to Nearctic and Paleartic B. transfuga worms, which have an average of 66 (Sprent, 1968). Other morphologic features were similar to B. transfuga, including overall length, a stout appearance, salient cervical alae, similar length spicules, and a rounded posterior margin of the pre-cloacal area (with B. venezuelensis having a “little process” on this margin) (Pérez Mata et al., 2016). Molecular analysis supported the separation of B. venezuelensis as a separate species. Combined ITS-1 and ITS-2 sequences were only 91.8% and 90.6% similar to B. transfuga and B. Schroederi, respectively (Pérez Mata et al., 2016). There were also three nucleotide differences in the highly conserved region of 5.8S rDNA that differentiated B. venezuelensis from the two ursid-associated species and other Baylisascaris spp. Phylogenetic analysis of both ITS regions included B. venezuelensis in a clade containing the other two ursid-associated species along with B. ailuri from red panda (Pérez Mata et al., 2016).

A few instances of previously detected ascadir eggs in fecal examinations of captive and free-ranging spectacled bears may represent B. venezuelensis infections (Schaul, 2006; Figueroa, 2016). Eggs designated as “roundworm” eggs were present in 9/25 (36%) of spectacled bear fecal samples from zoos across the United States, although it is impossible to determine if this is B. venezuelensis or native B. transfuga acquired from other bears in the captive environment (Schaul, 2006). Ascadir eggs resembling those of Baylisascaris or Toxocara were reported in 6/28 (21%) of T. ornatus scats from northern Peru, but measurements of the eggs were not provided (Figueroa, 2015). It is currently unknown if B. venezuelensis is usually pathogenic for spectacled bears. The type host is believed to have died from the nematode infection, but most previously reported positive spectacled bears were presumably asymptomatic. Although most Baylisascaris spp., including B. transfuga, rarely cause mortality in their definitive hosts, B. Schroederi from pandas is a major cause of morbidity and mortality so additional research on the potential risk of B. venezuelensis to spectacled bears is needed (Jiang et al., 2005).

The finding of a new, seemingly valid Baylisascaris species in a relatively isolated population of ursids further supports the idea that “B. transfuga” represents an assemblage of species globally, and highlights the need for further molecular and morphologic work to characterize possibly cryptic species. Field surveys are also necessary to determine the prevalence as well as definitive and paratenic host range of this new tropical species.

2.3. Baylisascaris laevis

Baylisascaris laevis uses rodents instead of carnivores as definitive hosts that makes it unique among the other Baylisascaris spp. in the New World (Berry, 1985). The parasite was first described in 1856 by Leidy as Ascaris laevis from naturally infected groundhogs (Marmota monax). Later it was reassigned to the genus Baylisascaris (Sprent, 1968).

Among the New World Baylisascaris species, B. laevis is generally smaller and wider than other species of Baylisascaris (Table 1). This species also has the smallest spicules and largest dorsal lip compared to other Baylisascaris spp. (Babero, 1960a; Sprent, 1968). Other differences involve the posterior end of the male and female worms with the female B. laevis tail abruptly tapered to a sharp point and the male tail narrowed mid-tail and appears swollen at the end (Tiner, 1951; Berry, 1985). Other diagnostic features included indistinct knobby protrusions near the cloacal opening of males (Tiner, 1951). The external genitalia of female B. laevis more anterior compared to B. columaris and B. procyonis (Berry, 1985).

2.3.1. Host range

The most commonly reported definitive host of B. laevis is the groundhog, a member of the Sciuridae family. Infections in other Sciridae hosts have been reported in the Alaska marmot (M. broweri), hoary marmot (M. coligata), yellow-bellied marmot (M. flaviventris), Olympic marmot (M. olympus), California ground squirrel (Otospermophilus beecheyi), Barrow ground squirrel (Spermophilus parryi barrowensis), Richardson’s ground squirrel (U. richardsonii), and long-tailed ground squirrel (Urocitellus undulatus) (Berry, 1985).

2.3.2. Differences in life cycle compared to other Baylisascaris spp.

B. laevis is the only member of the genus that has a strictly monoxenous life cycle with the apparent loss of a paratenic host during evolution. Development from L2 to adult occurs entirely in the sciurid definitive host (Berry, 1985). Unlike other Baylisascaris spp., larvae which migrate throughout the body of paratenic hosts, B. laevis only migrate within the liver and lungs of their hosts (Babero, 1960b).

When larvated eggs are ingested, L2 hatch and migrate to the liver by 10–12 DPI and develop into L3. These larvae migrate to the lungs where they molt into L4 that are coughed up and swallowed. Once in the small intestine they continue to develop into adults within the wall of the small intestine (Babero, 1960b). Adults enter the intestinal lumen, mate, and produce eggs that are then shed in feces. Reinfestation likely occurs when embryonated eggs adhering to fur are ingested during grooming (Berry, 1985). B. laevis can produce liver lesions in its sciurid host (Tiner, 1953b).

2.3.3. Ecology and epidemiology

Although the distribution of known B. laevis sciuriid hosts extends throughout North America, the parasite has only been reported in New York, Pennsylvania, California, and Alaska. Outside the United States it has been reported in southern Ontario and Saskatchewan, Canada (Berry, 1985). Further surveillance is needed to characterize the distribution of B. laevis in North America.

A few studies have been conducted on the seasonality of B. laevis infectious. During a 2 year study in southern Ontario, Canada, B. laevis prevalence peaked in September, with intensity showing similar seasonal variation (Berry, 1985). Prevalence was lowest during winter months and increased during the spring, similar to the annual cycles observed in B. columaris and B. procyonis (Berry, 1985). This seasonality of B. laevis seems to be primarily driven by feeding habits; groundhogs and ground squirrels continually feed throughout the spring and summer, and then in the fall begin to
consume less in preparation for hibernation. In the winter, absence of (Young and Sims, 1979) and physiological changes accompanying hibernation, such as lowered temperature, heart and metabolic rate of hosts, likely prevents B. laevis from developing if acquired late in the year (Babero, 1960b). Despite lower rates of shedding in the winter, eggs can persist in the environment because they are resistant to sub-zero temperatures. Most (94%) non-embryonated eggs survived –10 C temperatures after exposure for 10 days, while >70% of embryonated L3 eggs survived –10 C temperatures for 16 days (Berry, 1985). Also, eggs deposited in the environment are often protected from extreme temperatures because they are covered by leaf litter and snow or in subterranean burrows (Berry, 1985).

2.3.4. Experimental infections

A variety of species have been assessed as experimental hosts for B. laevis using experimental infections. Babero (1959) conducted oral infection trials to assess susceptibility and pathology of B. laevis infection in eleven species including laboratory mice, laboratory rats (Rattus norvegicus), cotton rats (Sigmodon hispidus), hamsters, guinea pigs (Cavia porcellus), thirteen-lined ground squirrel (Ictidomys tridecemlineatus) and Franklin ground squirrels (Poliocitellus franklinii), opossums (Didelphis virginiana), groundhogs, domestic cats, and raccoons. Many of these experimental hosts were examined of infection via necropsy, although details on the duration of infection and stages recovered are not given so interpretation is difficult.

Some inoculated hosts developed disease, including a groundhog with signs of pneumonia due to larva migration. Two guinea pigs, who died ~40 days post infection, exhibited dyspnea, bloody stools, ataxia and emaciation. Granulomatous liver lesions were frequently observed, sometimes containing L2 larval sheaths, as stools, ataxia and emaciation. In the winter, absence is needed to confirm the identity of parasites from North American badgers and their conspecificity with B. melis from European badgers.

Baylisascaris melis can cause larva migrans in experimentally infected rodents. Tiner (1953a) fed 2000–3000 eggs collected from a naturally-infected badger in Wyoming to four rodent species: ground squirrel (Citellus armatus), laboratory mice, deer mice (Peromyscus maniculatus) and guinea pigs. Only the ground squirrels (5 of 7 infected) developed neurologic disease; however, larvae were found in the brains of the deer mice and encapsulated in the skeletal muscle of all four species (Tiner, 1953a). In one group experiment, larvae were recovered from the lungs and were widely distributed in the skeletal muscle, with greatest abundance in the intercostal spaces under the parietal pleura of the diaphragm. In P. maniculatus, B. melis larvae were encapsulated in the mesentery of the small intestine, on the epidermal surfaces, and in the brain on
3 DPI (Tiner, 1953b).

Although B. melis can cause central nervous system disease in experimentally-infected rodent species, there have been no confirmed cases of natural B. melis infections in wild rodents (Boyce et al., 1988; Kazacos, 2001; Tiner, 1953a,b). However, neurologic cases due to Baylisascaris sp. diagnosed in ground squirrels and other rodents in regions where the badger ranges overlap with raccoons could be due to B. melis (Kazacos, 2001). In future cases, identification of larvae in these cases using molecular techniques is needed to better understand the role of non-B. procyonis species in cases of neurologic disease in wildlife. Also, serum from mice infected with B. melis cross-reacted with larval excretory-secretory antigens from B. procyonis (Boyce et al., 1988), so it is important to consider B. melis as a possible etiologic agent of hosts with antibodies to Baylisascaris spp. in areas where badgers and raccoons are sympatric.

2.6. Baylisascaris potosis

A novel Baylisascaris species, B. potosis, was recently described in kinkajous (Potos flavus). Type specimens were collected from captive kinkajous that originated in Cooperative Republic of Guyana (Tokiwa et al., 2014). This species is morphologically similar to B. procyonis but was described as a new species based on genetic analysis of several gene targets (i.e., internal transcribed spacer (ITS) 2 region, 28S rRNA gene, and COX1 gene) (Taira et al., 2013; Tokiwa et al., 2014). Kinkajous are common exotic pets in the United States and other countries. Previously, kinkajous were reported as a host of B. procyonis, both in the wild in Columbia and in captivity in the United States and Japan (Overstreet, 1970; Kazacos et al., 2011; Taira et al., 2013; Parzansky, 2015). Another possible host, the bushy-tailed olingo (Bassaricyon gabbii) passed a male Baylisascaris (reported as B. procyonis) after being fed eggs from a naturally-infected kinkajou from Columbia; however, it is not known if the olingo was infected prior to the experiment (Overstreet, 1970). However, since the description of B. potosis, these reports are questionable, and where possible should be confirmed with molecular data.

To evaluate possible paratenic hosts that can develop larva migrans, Tokiwa et al. (2015a), experimentally inoculated Mongolian gerbils. Exposure of gerbils to 100–4000 embryonated eggs resulted in VLM, but no larvae were found in the brain. A squirrel monkey (Saimiri sciureus) inoculated with 10,000 B. potosis eggs did not develop clinical signs or gross lesions, although a few migrating larvae were recovered from liver and kidney tissues (Tokiwa et al., 2015b). Another squirrel monkey inoculated with 100,000 eggs in the same trial developed gross lesions, including liver congestion, pulmonary edema, and abundant intestinal granulomas. Small granulomatous lesions containing larvae were found in the outer layers of the cerebral cortex, without deeper invasion as is typical of B. procyonis. This animal was found dead at 30 DPI, but the animal lacked clinical signs and the authors state that no cause of death was determined. However, pulmonary edema, liver congestion, and nodular lesions containing non-degenerate larvae along the intestine were found at necropsy (Tokiwa et al., 2015b). Based on these preliminary trials, it appears that B. potosis can cause larva migrans in rodent and primate hosts, although the pathogenicity and capacity for neural invasion appears less than that of B. procyonis or B. columnaris.

Because of the recent description of this parasite and the paucity of surveillance in possible hosts in South America apart from a single infected individual, little is known about the natural history of B. potosis. Interestingly, raccoons were recently confirmed to have B. procyonis infections, based on sequence analysis, in Costa Rica (Baldi et al., 2016). It appears that B. procyonis and B. potosis are sympatric in procyonids in Central America, highlighting the need for additional research to understand these closely related parasites that may share hosts.

3. Molecular and diagnostic approaches to the study of Baylisascaris

3.1. Molecular epidemiology of Baylisascaris spp.

Microscopy has been traditionally used to identify Baylisascaris spp. based on adult morphological characters although some species can be difficult to distinguish, especially if only immature worms are found. However, because of the similarity among the

### Table 5

| Host species                     | Location                  | No. infected/No. examined (%) | Source                  |
|---------------------------------|---------------------------|------------------------------|-------------------------|
| American marten (Martes americana) | Manitoba, Canada          | 1/139 (0.7)                  | Poole et al., 1983      |
|                                 | Alaska, USA               | 1/141 (0.7)                  | Scranton 1986           |
| Wolverine (Gulo gulo)           | Washington, USA           | 4/78 (5)                     | Hoberg et al. 1990, a   |
| Fisher (Martes pennanti)        | Northwest Territories, Canada | 17/80 (21)               | Addison and Boles 1978  |
|                                 | New Brunswick, Canada     | NG                           | Dick and Leonard 1979   |
| Pacific marten (Martes curinae) | Idaho, USA                | 52/162 (32)                  | Dick and Leonard 1979   |
| Martes americana, Martes pennanti | Ontario, Canada          | 6/17 (35)                    | Marshall 1942, b        |

NG—Not given.

a Unidentified larvae authors suggested could be B. devosi were found in digestions of hind limb musculature of 10 martens in addition to the 23 adult nematodes identified as B. devosi in study.
b Identified as Ascaris columbiana.

### Table 6

| Location          | No. infected/No. examined | Source             |
|-------------------|---------------------------|--------------------|
| Minnesota         | 1/8                       | Erickson 1946      |
| Iowa              | 29/NG                     | Wittrock and Ulfner 1974 |
| North Dakota      | 6/17                      | Leiby et al., 1971 |
| Kansas            | 10/30                     | Pence and Dowler 1979 |
| Wisconsin         | 2/4                       | Morgan 1943        |
| South Dakota      | 13/100                    | Jense 1968         |
| Colorado          | NG                        | Leiby 1961         |
| Wyoming           | 1 roadkill badger, 1 captive badger | Tiner 1953a |

NG—Not given.

a Identified as Ascaris columbiana but are assumed to be B. melis.  
b 2 of the 8 badgers had been in captivity for 2 years.
sizes of eggs in feces or larvae in tissues, molecular markers have been developed to facilitate identification. For example, multiple single nucleotide polymorphisms (SNPs) in mitochondrial and nuclear gene sequences of *B. columnaris*, *B. procyonis* and *B. transfuga*, have been used to develop species-specific diagnostic molecular markers for rapid identification of different *Baylisascaris* species (Blizzard et al., 2010; Testini et al., 2011; Franssen et al., 2013). Studies have also characterized the genetic diversity, investigated the population structure and the phylogenetic relationships among *Baylisascaris* species, which is important for understanding the zoonotic potential and host specificity of these parasites. To discuss the phylogenetic relationships among the *Baylisascaris* spp. that occur in the New World in a broader context, this section includes data published on *Baylisascaris* spp. from Asia and Europe and *Baylisascaris procyonis* from raccoons.

3.1.1. Phylogenetic relationships

Based on analysis of numerous genetic targets (Table 7), *Baylisascaris* is most closely related to the genus *Ascaris*. The entire mitochondrial genome has been sequenced for four species—*B. transfuga*, *B. ailuri*, *B. Schroederi* and *B. procyonis* (all samples from China) (Xie et al., 2011a, 2011b; Li et al., 2012). Phylogenetic analyses of these mitochondrial genomes and concatenated partial mitochondrial and nuclear genes (12S rRNA, 18S rDNA and 28S rDNA) provide the strongest support for the relatedness of the genus *Baylisascaris* with *Ascaris* and other members of the order *Acaridida* (Xie et al., 2011a, 2011b; Li et al., 2012).

Within the *Baylisascaris* genus, phylogenetic relationships have been investigated using several molecular targets (i.e., numerous mitochondrial genes, nuclear 5.8S, and second internal transcribed spacer (ITS-2) rDNA sequences). The three ursid-specific *Baylisascaris* species (*B. transfuga*, *B. ailuri* and *B. Schroederi*) are more closely related to each other than to *B. procyonis* and *B. ailuri* is more similar to *B. transfuga* than to *B. Schroederi* (Xie et al., 2011a, 2011b; Li et al., 2012). Evidence from nuclear 5.8S and ITS-2 rDNA sequences also showed higher genetic similarity between *B. transfuga* and *B. Schroederi* compared to *B. procyonis* (Zhao et al., 2012).

Although no molecular data are available for *B. laevis*, *B. melis* or *B. devosi*, mitochondrial and nuclear genes of *B. columnaris* and *B. potosis* have recently been characterized and their phylogenetic relationship with other *Baylisascaris* species has been examined. The first phylogenetic analysis of *B. columnaris* from pet skunks in Europe showed closer affinity to *B. procyonis* compared to *B. transfuga* (Franssen et al., 2013) based on mitochondrial cytochrome *c* oxidase 1 and 2 (CO1 and CO2), ribosomal ITS1-5.8S-ITS2 and ribosomal 28S genes. This result was expected because *B. columnaris* was previously shown to be very similar to *B. procyonis* based on partial mitochondrial CO2 gene sequences (Dangudoubiyam et al., 2009). Phylogenetic analyses of the mitochondrial CO1 and ITS2 rDNA gene sequences showed that *B. potosis* had high genetic similarity to *B. procyonis* and *B. columnaris* (Tokiwa et al., 2014).

A comprehensive phylogenetic assessment of different *Baylisascaris* species at common gene targets would facilitate a better understanding of genetic similarities/differences between the different species. Given that many species of *Baylisascaris* span a very wide geographic scale, genetic differences likely exist among these populations suggesting the existence of cryptic species. For example, *B. melis* is endemic to both Eurasia and the Americas and *B. transfuga* similarly has a large geographic and host range. The morphologic variability in *B. transfuga* also should be examined using molecular tools to assess if these indicate the presence of multiple species. Genetic studies are critically needed to evaluate species validity and both fine-scale and broad-scale geographic variability for all *Baylisascaris* species.

3.1.2. Population structure

Genetic markers are widely used to assess population structure and provide important insights into host-parasite transmission dynamics. For example, giant panda (*Ailuropoda melanoleuca*) populations were genetically distinct across the three mountain ranges in China but *B. Schroederi* were not, suggesting little co-evolution between hosts and parasites and high levels of parasite gene flow (Zhou et al., 2013; Xie et al., 2015). High genetic variation within the parasite populations on each mountain range was observed, but the lack of population diversity across the mountain ranges suggested a homogenous parasite population, based on the complete mitochondrial *cytb*, *atp6* and *cox1* gene targets. In contrast, use of microsatellite markers revealed two genetic clusters in *B. procyonis* across the Grand River in Western Michigan, USA (Sarkissian et al., 2015). Lack of population structure in *B. Schroederi* parasites across the mountain ranges in China indicates the fast evolving rate of parasites compared to their hosts and microsatellite markers may be able to further confirm if there is any recent genetic divergence between the parasite populations. Thus, choice of appropriate genetic marker is important while assessing parasite population structure and understanding host-parasite evolutionary dynamics.

Low genetic diversity was found within *B. columnaris* in the Netherlands. Multi-locus genetic analysis revealed four distinct genotypes, possibly owing to the differences in the host or geographic origin of these parasites (Franssen et al., 2013). However, a wider sampling of infected hosts from other geographical regions is warranted to reveal the true genetic diversity of these parasites.

3.2. Diagnostic considerations

As discussed throughout this review, accurately distinguishing species based only on morphologic characteristics can be a challenge. Not only does considerable overlap in morphometry exist (Table 1), but host species associations may not be as strict as often assumed as limited experimental data suggest that *B. columnaris* can infect raccoons and *B. procyonis* and *B. devosi* can infect skunks (Berry, 1985; Spret, 1953a). Although the prevalence of these cross-infections in naturally-infected hosts is not understood, parasite species identity should not be assumed based on host species. When possible, molecular identification of species should be used for species confirmation of eggs, larvae, and possibly adult nematodes if only female or immature worms are present. Until this is widely implemented, our understanding of host specificity among *Baylisascaris* spp. will remain limited.

Additionally, current serologic methods cannot distinguish between species of *Baylisascaris* causing larva migrans in paratenic hosts. Antiserum from animals infected with different *Baylisascaris* spp. all show reactivity to crude *B. procyonis* excretory-secretory (ES) antigen fractions as well as a recently developed recombinant antigen (BpRAG–1) (Boyce et al., 1988; Dangudoubiyam et al., 2010). Further work is needed to determine if species-level differences in ES or other antigen targets exist, and if these differences would be sufficiently different to allow the development of species-specific serodiagnoses. This is a potentially difficult goal as serologic differentiation between two other related ascarids, *Toxocara canis* and *Toxocara cati*, has not been successful with current platforms, and these are both important zoonotic parasites (Poulsen et al., 2015). Therefore molecular identification of recovered larvae from paratenic hosts, if possible, is the ideal method of confirming species. Some serologic assays may be useful in determining exposure to parasites in the genus, given that the target antigen is not cross-reactive with other ascarids.
Table 7
Summary of different PCR primers available for identification of Baylisascaris species.

| Target gene | Length of the target gene | Species | Primer | Reference |
|-------------|----------------------------|---------|--------|-----------|
| ATPase subunit 6 (αt6) | 600bp | B. schoederi | F (5'-CCGGCATGTCATCCTCAGG CT-3') | Xie et al., 2015 |
| cytochrome oxidase c subunit 1 (CO1) | 413bp | R. procyonis, B. transfuga, C. columnaris | R (5'-CCCAAGCTTTATGTTGGGAGG 3') | Franssen et al., 2013 |
| cytochrome oxidase c subunit 2 (CO2) | 1578bp | B. schoederi | F (5'-TTTAGGCTGGAATCAGGTTCTG-3') | Xie et al., 2015 |
| mitochondrial | 1500bp | B. schoederi | Cyb-1 (5'-CCCAAGCTTTATGTTGGGAGG 3') | Zhou et al., 2013 |
| 12s rRNA | 499bp | B. schoederi, R. procyonis, B. transfuga, C. columnaris | F (5'-CCGGGAGCGGAAATGACAAAA 3') | Li et al., 2012 |
| 18s rDNA | 1708bp | B. schoederi, R. procyonis, B. transfuga, C. columnaris | F (5'-TGATCCCTCTGACG 3') | Li et al., 2012 |
| 28s rDNA | 751bp | B. schoederi, R. procyonis, B. transfuga, C. columnaris | F (5'-CCGGGAGCGGAAATGACAAAA 3') | Li et al., 2012 |
| ITS1-5.8S-ITS2 | 630-650bp | B. schoederi, R. procyonis, B. transfuga, C. columnaris | See reference for numerous sets of primers. | Xie et al., 2011a; Xie et al., 2011b |
| Complete mt genome-(αt6, CO1–CO3, cytb, nad1–nad4L, 22 transfer RNA (trn) Genes (small (rrnS) and large (rrnL) subunits) | | | | |
| ITS2 rDNA | 301 bp | B. transfuga | F (5'-TTAGTAATTTTCCACCATGCG-3') | De Ambrogi et al., 2011 |
| 3' end of the ITS-1, complete 5.8s and ITS-2, and the 5' end of 28s rDNA | 700bp | B. schoederi | F (5'-AAGGTTGAGGAAGACCCCTGCT-3') | Zhao et al., 2012 |
| ITSs (18s and 28s) | 1177bp | B. transfuga | F (5'-TTAGTAATTTTCCACCATGCG-3') | Testini et al., 2011 |

* Different length in different parasites due to insertions and tandem repeat.

4. Conclusion

The genus Baylisascaris is diverse with importance to wildlife, domestic animal, and public health. However, many knowledge gaps exist regarding species other than B. procyonis, which is largely driven by a scarcity of contemporary surveys and application of molecular tools to investigate the ecology of these parasites Field studies elucidating important life history characteristics are critically lacking for these other, “neglected” Baylisascaris spp. Ideally, these future field efforts will incorporate modern molecular approaches along with traditional morphologic examination to better ascertain species diversity, species validity, host range, and disease caused by these parasites in wild definitive and paratenic hosts.

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