

## Short Note

# Infection of the invasive slug *Arion vulgaris*, by the nematode, *Alloionema appendiculatum*, and possible host-switching

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The ‘Spanish’ slug *Arion vulgaris* Moquin-Tandon, 1855 (Gastropoda, Arionidae) is an alien pestiferous slug with an outstanding adaptive ability and is the only terrestrial gastropod in the list of 100 worst invasive species of Europe (Rabitsch, 2006; Zemanova *et al.*, 2016). Its distribution expanded greatly in the last decades due to the changes in climate, trade and lifestyle (Hatteland *et al.*, 2013; Zajac *et al.*, 2017; Balashov *et al.*, 2018). Recently, its phylogenetic history was reconstructed (Zemanova *et al.*, 2016; Zajac *et al.*, 2020) and the origin of the species, which was long argued, has been clarified. The first genome assembly for the species was also presented (Doğan *et al.*, 2020).

Recently, Spanish slugs have established in Moscow green areas. Based on molecular-phylogenetic analysis, we showed that *Arion vulgaris* inhabiting parks of Moscow city belonged to several lineages originating from Central Europe (Ivanova *et al.*, 2022). During the survey of 2021, 15 species of land gastropods were examined for the presence of parasitic nematodes. At the time, *A. vulgaris* was the only species infected by *Alloionema appendiculatum* (Schneider, 1859). This nematode has a life cycle including a parasitic stage in the foot muscle of the gastropod and a free-living stage living on gastropod cadavers and in soil (Mengert, 1953; Morand, 1988; Nermut *et al.*, 2015, 2019). The nematode is a common parasite of arionid slugs but capable of infecting some other gastropod taxa (Agriolimacidae, Helicidae, Hygromiidae, and Succineidae) (Chitwood & McIntosh, 1934; Morand *et al.*, 2004), though with very low prevalence. Different arionid species showed variable susceptibility to the nematode (2 to 100%) (Mengert, 1953; Morand *et al.*, 2004).

The alien origin of *A. appendiculatum* in *A. vulgaris* in Moscow was confirmed by the sequencing of ITS and *COI* 1 markers and showed that *A. appendiculatum* that infected three Moscow populations of Spanish slugs were identical to populations reported from Central Europe. It was assumed that the infection may spread due to the presence of suitable gastropod hosts, and consequently, the co-introduced parasite may become co-invasive (Ivanova *et al.*, 2022).

Since 2021, we monitored the presence of *A. appendiculatum* in gastropods in the Main Botanical Garden (MBG) in Moscow (nurseries and grounds). In regard to *A. vulgaris*, prevalence and intensity of *A. appendiculatum* infection was high, reaching 100% (prevalence) and several hundred individuals (intensity). In 2022, no *A. appendiculatum* was discovered, other than in *A. vulgaris* gastropods. In 2023, for the first time we collected specimens of arionid slugs (*Arion subfuscus* and *Arion fasciatus*) infected by *A. appendiculatum*.

Slugs *A. subfuscus* (Draparnaud, 1805) and *A. fasciatus* (Nilsson, 1822) are species native to Europe with wide distribution area including North America (Kerney & Cameron, 1979; Byers, 2002; Sysoev & Schileyko, 2009). These slugs live in natural and ecotone habitats, such as woodland, grassland and road verges, locating in leaf litter. Both species are almost omnivorous feeding mostly on decaying plant and animal material. They rarely reach high population densities and are not commonly associated with crop damage, although *A. subfuscus* can be an occasional crop pest (Kerney & Cameron, 1979; Byers, 2002) in countries where it is introduced. At the same time, Howlett (2012) lists both species as key pest slug species of temperate regions.

In Moscow parks, they are common but never abundant and do not cause significant damage to nursery stock. In the summer of 2023, only 12 specimens of *A. subfuscus* and 23 specimens of *A. fasciatus* were found in four sampling visits at the same nursery in MBG where they were collected along with numerous Agriolimacidae slugs (four species), which were never found infected by *A. appendiculatum*. Five specimens of *A. fasciatus* (21.7%) and two of *A. subfuscus* (16.7%) were found infected with *A. appendiculatum*. In the early summer, each infected slug specimen contained a single parasitic juvenile located in the neck region of the host. Slugs sampled in September contained much larger load of *A. appendiculatum*, and two *A. fasciatus* specimens were collected dying and died 2 days after sampling. According to Nermut *et al.* (2019), *A. appendiculatum* typically leave their hosts (*A. vulgaris*) late in the autumn, although mass release of the nematode was also observed in earlier months.

The relationships of *A. vulgaris* with parasitic nematodes were studied more than that of any other slug species due to invasive status of this slug species. According to a number of studies (Ross *et al.*, 2015; Nermut *et al.*, 2019; Laznik *et al.*, 2010, Antzée-Hyllseth *et al.*, 2020; Filipiak *et al.*, 2020), *Arion vulgaris* is a typical host for *A. appendiculatum*. Laznik *et al.* (2010) implied that *A. appendiculatum* can have pathogenic effect on the slug and even cause its death. Nermut *et al.* (2019) did not support the view and assumed that the nematode does not affect its fitness in the wild. Cabaret *et al.* (1988) reported the pathogenic effect of *A. appendiculatum* in heliculture. Antzée-Hyllseth *et al.* (2020) showed the ability of *A. vulgaris* to withstand a remarkable load of *A. appendiculatum* and suggested that *A. vulgaris* might owe its success to its tolerance to parasites. Transcriptomic and proteomic studies by Bulat *et al.* (2016) revealed a number of certain toxic proteins (defensins) in *A. vulgaris* which the authors of the study suggested may be responsible in such a success of this slug species. According to Antzée-Hyllseth *et al.* (2020), *A. fasciatus* bore considerably lower load of *A. appendiculatum* in the natural infection compared with *A. vulgaris*.

However, what impact *A. appendiculatum* can have on the new hosts remains unknown. As a new element of the gastropod parasite fauna, it might have an ability to alter host-parasite relationships (Westby *et al.*, 2019; Romeo *et al.*, 2021; Katahira *et al.*, 2022) of related gastropod species. The local gastropod fauna of the city green spots already contains a number of non-native species and currently undergoes invasions by yet more alien

species. Introduced European snail species, such as *Arianta arbustorum*, *Cepaea* spp., *Helix* spp., and agriolimacid slugs from Caucasus (*Krynickillus melanocephalus*, *Deroceras caucasicum* and *Boetgerilla pallens*) (Tappert, 2009; Shikov, 2016), have already become residential in parks of Moscow city and compete with native species. Out of all resident gastropods, the native arionids are the most susceptible to *A. appendiculatum* invasion and will be targeted in host-switching if it happens.

To assess the further spread of *A. appendiculatum* and its impact on local gastropods, monitoring will be continued.

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