BioInvasions Records (2023) Volume 12, Issue 1: 321–329

Rapid Communication

First records of *Scinax ruber*, *Podarcis siculus*, *Podarcis ionicus* and their parasites in Latvia: fruit trade is an intercontinental alien herpetofauna and parasitofauna invasion vector into Europe

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Citation: Pupins M, Martinez-Silvestre A, Arribas O, Čeirāns A, Kirjusina M (2023) First records of *Scinax ruber*, *Podarcis siculus*, *Podarcis ionicus* and their parasites in Latvia: fruit trade is an intercontinental alien herpetofauna and parasitofauna invasion vector into Europe. *BioInvasions Records* 12(1): 321–329, https://doi.org/10.3391/bir.2023.12.1.29

Received: 27 September 2021
Accepted: 30 November 2022
Published: 13 February 2023

Handling editor: Rui Rebelo
Thematic editor: Tim Adriaens

Abstract

Expanding the trade of tropical fruits in an increasingly globalized world increases the likelihood of unintentional importation of viable herpetofauna and their parasites from other countries and even continents, since the conditions for growing and transportation of fruits (humidity, temperature) can often be tolerated by exotic amphibians and reptiles. Here we describe our findings of two reptiles, *Podarcis siculus campestris* and *P. ionicus* (formerly *P. tauricus ionicus*), and an amphibian, *Scinax ruber*, in boxes of tropical fruits in Daugavpils city, Latvia, European Union. In our parasitological survey of *S. ruber*, we found two groups of endoparasites: nematoda (*Physaloptera* sp.) and trematoda (*Travtrema* aff. *stenocotyle* mtc.). In *P. ionicus* intestine we found acanthocephalan *Centrorhynchus* sp. larvae. All these parasites were detected in Latvia for the first time. The estimated minimal direct distance of the trans-oceanic relocation of *S. ruber* and its parasites from its natural distribution centre in Suriname to its finding place in Latvia exceeds 10 600 km.

Key words: trans-oceanic bioinvasion vector, fruit trade threat, alien parasites, Lacertidae, Hylidae

Introduction

Alien species may represent a major threat to native animals and plants when they are accidentally or deliberately introduced into natural environments (Crowl et al. 2008). Of the 55 amphibian and reptile species successfully introduced into Europe, 12 species have been reported to have adverse effects on ecosystems (Kark et al. 2009). Latvia has already faced threats from invasive herpetofauna, with an overwintering exotic turtle, *Trachemys scripta* (Pupins 2007; Pupins and Pupina 2011), which is dangerous for rare native turtle *Emys orbicularis* (Pupins et al. 2017), due the direct impact and new parasites species (Polo-Cavia et al. 2011; Iglesias et al. 2015). Several turtle species are potential invaders from pet stores (Kopecky et al. 2013; Pupina and Pupins 2016), and thus the alien herpetofauna problem requires attention in Latvia, especially as it lies with the northernmost range of several rare European herpetofauna species (Kuzmin et al. 2008; Pupins et al. 2017; Čeirāns and Pupins 2019; Čeirāns et al. 2020) and under impact of global climate change (Nekrasova et al. 2021).

One important effect of alien herpetofauna introductions is the importation of new parasite species and pathogens far from their native range, and their subsequent transfer to new hosts and thus alien parasite invasions. Alien herpetofauna species can also act as new vectors and hosts for native parasites. Such invasions have been described in European turtles (Soccini and Ferri 2004; Hidalgo-Vila et al. 2009; Iglesias et al. 2015), while ranavirus has been found in introduced newts (Martinez-Silvestre et al. 2017), *Serpinema* parasite in Asian and American invasive turtles, and Mycoplasma and herpesvirus in introduced *Trachemys scripta* turtles in Spain (Martinez-Silvestre et al. 2015; Hidalgo-Vila et al. 2020). Perhaps the most devastating example is the worldwide spread of amphibian chytridiomycosis (Fisher and Garner 2020); the latter has also been recorded on *Pelophylax* frogs in Latvia (Kulikova et al. 2022).

Although the targeted importation of invasive species in EU member states is regulated by EU Regulation 1143/2014 on Invasive Alien Species and national legislations, the unintentional importation of alien animals with food or other goods is still possible because complete control over every imported box and container is not feasible. Expanding the trade of tropical fruits increases the likelihood of unintentional importation of viable herpetofauna from other countries or even continents (Martinez-Silvestre et al. 2019), since the conditions for growing and transportation of fruits (such as humidity and temperature) can meet the environmental needs of herpetofauna.

Materials and methods

In our search for the unintentional importation of exotic herpetofauna species, we presented several informative reports about the alien species to the people of Latvia through the medium of TV, radio and newspapers, and asked them to contact us immediately after discovering an unknown species, or unusual or exotic specimen of an amphibian or reptile. Following any contact, we visited the site and, if we managed to catch the specimen, placed the animal in a well-ventilated plastic box and transported it to the laboratory. The specimens of findings 2 and 3 were euthanized in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes and FELASA under FELASA-certified specialist supervision (Guillen 2012). Full helminthological dissection of the animals was performed (Skryabin 1928; Pessier and Mendelson III 2009). This procedure included microscopy and visual inspection and parasitological examination of the removed internal organs, body cavity, spaces under the skin and limb muscle; after the study the material was deposited and stored in ethanol.

Table 1. Alien herpetofauna species and its helminths registered in the fruit-trade in Latvia.

Finding_#	Date	Location	Fruit	Origin	Herpetofauna species	Helminths found
1 (authors)	2012.04.05	Supermarket, fruit section, Daugavpils	tangerine	Italy	*Podarcis siculus campestris* (Rafinesque, 1810), Lacertidae, male (Figure 1A)	study has not been done
2 (authors)	2018.09.12	Small market, Spogi village, Augsdaugava district, Latvia	bananas	Republic of Suriname	*Scinax ruber* (Laurenti, 1768), Hylidae (Figure 2A)	*Physaloptera* sp. (in stomach, length: 3543.40 µm; width: 198.15µm) (Figure 2B); *Travtrema* aff. *stenocotyle* (four metacercaria in mesenterium, length: 607.54 µm; width: 297.19 µm) (Figure 2C);
3 (authors)	2020.04.21	Supermarket, Daugavpils, Latvia	oranges, bananas	Spain, Ecuador	*Podarcis ionicus* (Lehrs, 1902), Lacertidae, male, (Greek species) (Figure 1B)	*Centrorhynchus* sp. larva (in the intestine, length: 3320.94 µm; width: 570.92 µm) (Figure 1: C, D, E, F).
4 (message and photo, caught by local citizen)	2020.07.04	Service station for fruit trucks, Riga, Latvia,	unknown fruits	southern Europe	*Tarentola* sp., Gekkonidae, adult	study has not been done, we didn't get the specimen
5 (message and photo, caught by local citizen)	2020	Riga, Latvia	unknown fruits	unknown	*Lacerta* sp. *viridis complex*, Lacertidae, adult	died soon and many worm-like parasites were found by its owner, we didn't get the specimen

Results

In 2012 and 2020 we caught two lizards, in 2018 we caught a frog, and in 2020 we received two Facebook messages about lizards in fruit markets, but received only low quality photos. Data on the hosts found and detected parasites are summarized in Table 1. Finally, in 2016–2018 we received reports of but did not manage to catch two lizards and one frog reported to be in packages of fruits in minimarkets in Latvia.

Discussion

We have found three parasite taxa new for the helminth fauna of Latvia: a spirurid nematode *Physaloptera* sp., larval stages of plagiorchiid trematode *Travtrema* aff. *stenocotyle* and an acanthocephalan *Centrorhynchus* sp.; the nematode and the trematode are representatives of Neotropical fauna, while an acanthocephalan is a native taxon for Europe (Komarova et al. 2015).

Nematodes *Physaloptera* spp. use amphibians as paratenic hosts; they have been found in at least two families and more than ten species of Neotropical (South and tropical Central America) anurans (toads, frogs, other tailless amphibians). Their definitive hosts are small predatory mammals (including domestic cats and dogs) and snakes (González and Hamann 2008; Washabau and Day 2013; Velarde-Aguilar et al. 2014). Encysted infective larvae of *Physaloptera* spp. have been detected in several species of insects, including beetles, cockroaches, and crickets (de Quadros et al. 2014). Mice and frogs may be paratenic hosts of these nematodes (Tung

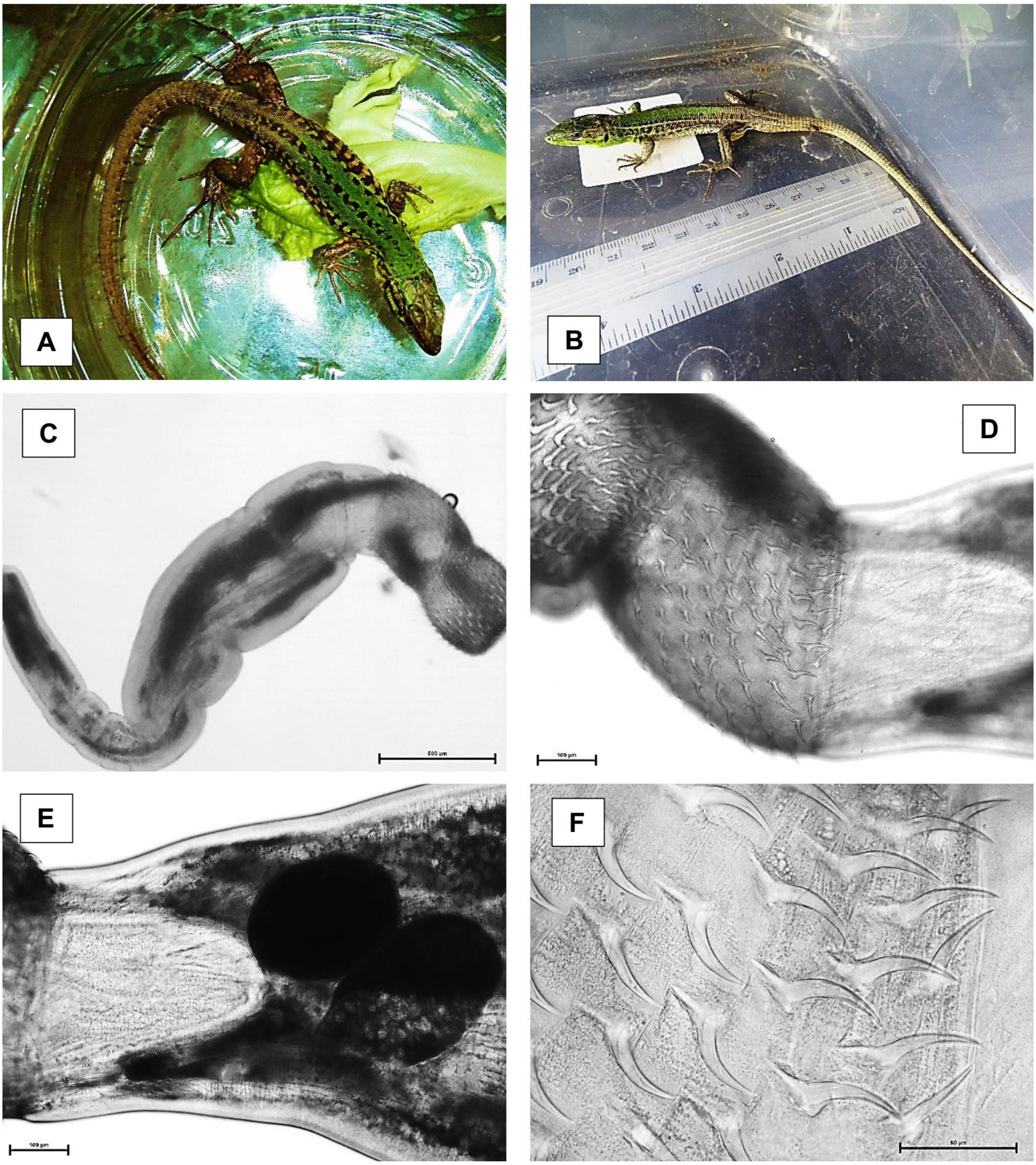

Figure 1. *Podarcis siculus campestris* (A). *Podarcis ionicus* (B) and its parasite acanthocephalan larva *Centrorhynchus* sp. (C, D, E) with characteristic thorny proboscis detail (F). Photo by M. Pupins (lizards) and M. Kirjusina (parasites).

et al. 2009; González and Hamann 2010; Klaion et al. 2011; Campião et al. 2014). *Physaloptera* species have also been reported from the gastrointestinal tract of nonhuman primates (Jaskoski 1960; Wong and Conrad 1972).

A plagiorchiid trematode *Travtrema stenocotyle* is often recorded in the Neotropical hylid frogs from the *Scinax* genus (Hamann et al. 2010), which act as second intermediate hosts, while the definitive hosts are snakes.

The acanthocephalan *Centrorhynchus* sp. larva in our study was found in the intestine of *Podarcis ionicus*. This helminth occasionally uses terrestrial

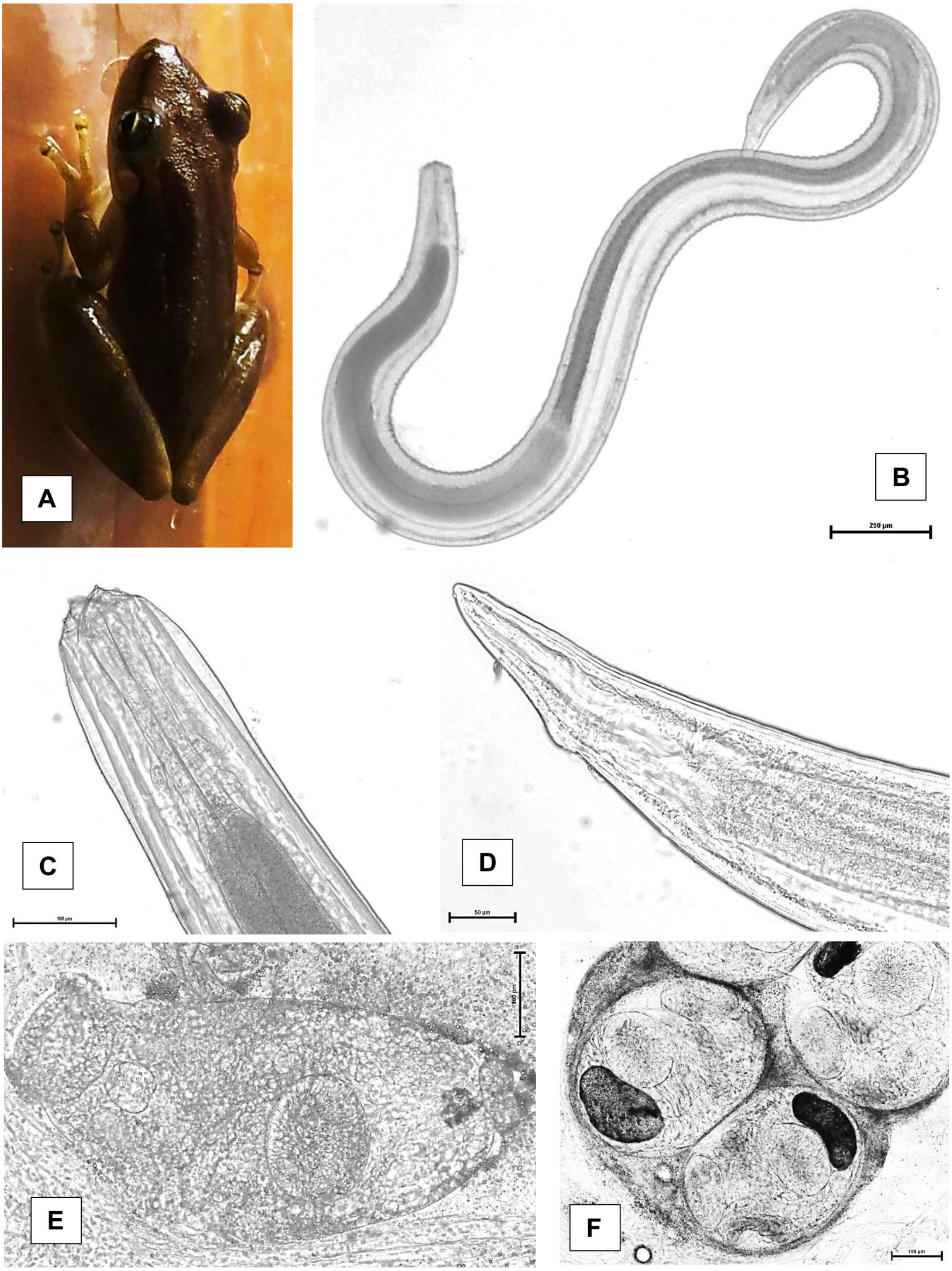

Figure 2. *Scinax ruber* (A), its parasites nematoda *Physaloptera* sp. (B, C, D) and metacercaria of *Travtrema* aff. *stenocotyle* (E, F). Photo by M . Pupins (frog) and M. Kirjusina (parasites).

reptiles, especially lizards, as paratenic hosts (Mader 2006), while predatory birds, especially some species of owls, are typical definitive hosts (Atkinson et al. 2008; Komarova et al. 2015).

The international fruit trade is a long-distance trans-oceanic and intercontinental invasion vector of new alien herpetofauna and their parasites in Europe. The direct minimum distance from the centre of the natural range of *S. ruber* in Suriname to its discovery in Latvia exceeds 10 600 km. *Podarcis siculus* is an invasive species and has native and introduced areas in Asia, Africa, North America and European countries. We estimate that it travelled ~ 1 800 km from its home range or possibly more than 8 000 km, if distributed from previously invaded territories in North America. All the herpetofauna specimens found were alive and in good condition, illustrating that the humidity and temperature conditions during the transportation of tropical fruits are tolerated by exotic herpetofauna species and their parasites and allow them to travel long distances, acting as a global parasite and invasion vector.

This indicates possibility of establishing of helminth populations well outside their native rage by the eating of unintentionally transported lizards or frogs by native predators, as it is suggested by the presence of a South-European acanthocephalan in *P. sicula* in our study, which is suitable potential prey for birds and domestic or wild mammals. Establishing of tropical parasites (such as found in hylid frogs from fruit stores) is less likely, but still possible. All the parasite species found by us are not pathogenic for humans (Hamann and González 2009). However, one was found in primates (Wong and Conrad 1972) and they can interact with the ecological networks (mainly the herpetofauna) of Latvian fauna, as demonstrated by the Mediterranean parasite *Serpinema* in *Trachemys scripta* and *Ocadia* (*Mauremys*) *sinensis* (Martinez Silvestre et al. 2015). In such cases, it is impossible to guess the final effect of such ecological disturbance. Global climate change could be an additional trigger for the success of thermophilic herpetofauna invasion into European countries (Nekrasova et al. 2021), including fruit-distributed species and their parasites.

On the basis of these case findings three main types of invasion of alien herpetofauna through the fruit trade were identified: 1) *Direct invasion* (from the importing country to the country of the final consumer or to intermediate countries); 2) *Cross invasion* (alien species move between imported fruits of different origins during transportation and storage, *cross*-contaminated the fruits boxes); 3) *Intermediate invasion* (invasion occurs in countries *intermediate* in the fruit logistics from wild populations living in these countries) (Figure 3). Identification of these three types of fruit-invasion can be useful both for the operative assessment of the origin of invasive herpetofauna and for the development of specific protective and preventive measures. So, for example, in the case of logistics of clear tropical fruits through intermediate warehouses in invasive-dangerous countries, to prevent *intermediate invasion*, it may be proposed to install special barriers around fruit storage warehouses. To prevent *cross invasion*, separate storage of fruit from different countries can be suggested.

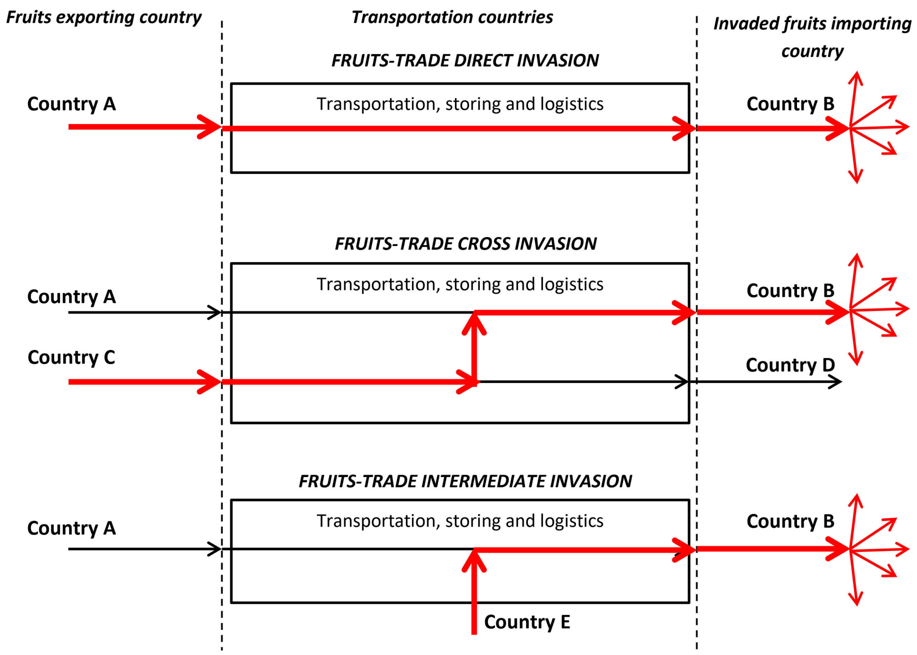

Figure 3. Three possible types of invading alien herpetofauna identified in this study: *Direct invasion*, *Cross invasion*, and *Intermediate invasion* (red arrows – way of distribution of the invading herpetofauna and its parasites by the fruit trade).

Genetic studies are thus necessary to identify the sources of invasion. In our study, no parasites dangerous to humans were found, but their long-distance and trans-continental fruit-trade spread in alien herpetofauna seems to us very possible and this requires special preventive measures at the national and international levels. The prevention of the fruit-trade-based invasion of alien herpetofauna and their parasites should include measures taken for all three types of invasion: in importing countries, in intermediate transporting countries, and in the end-consumer countries.

Of course our case study cannot be used to estimate the real magnitude of unintentional exotic species importation with tropical fruits, or the real probabilities of new parasite invasions. Still, it illustrates the possible risks of global long-distance fruit trade for native species and the risks of alien parasite release into new ecosystems, since in the vicinities of our findings are populations of both rare and widespread anuran species (Čeirāns et al. 2020) which could become paratenic or intermediate hosts of the recorded exotic parasite taxa, as well as rare turtle, snake (Pupins and Pupina 2015; Pupins et al. 2017) and lizard populations, which could become new definitive hosts and alien parasite reservoirs.

Acknowledgements

We are thankful to Alex Gurzhiy and Igor Zhidov for the identification of the Anura species. We thank for cooperation the projects # lzp-2021/1-0247 (Fundamental and Applied Research Projects); # 16-00-F02201-000002 (Rural Support Service of the Republic of Latvia) for using the mobile laboratory complex, Latgales Zoo and all respondents reporting alien species findings. We sincerely thank anonymous reviewers and the handling editor Dr Rui Rebelo for valuable suggestions improving the manuscript.

Authors' contribution

AM-S, MP, OA: research conceptualization; MP, MK, AM-S, OA: sample design and methodology; MK, MP, AČ: investigation and data collection; MK, MP, AM-S, OA, AČ: data analysis and interpretation; MP, AČ: ethics approval; AČ, MP, MK: writing – original draft; all co-authors: writing – review and editing.

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