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Occurrence of an invasive slug *Limacus flavus* (Stylommatophora: Limacidae) in the trees of an urban landscape in Kyiv city (Ukraine), with remarks on its colouration

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ABSTRACT. The importance of trees for an invasive Mediterranean cellar slug, *Limacus flavus*, was studied within one block of a residential area in Kyiv city (Central Ukraine) from spring of 2020 to spring of 2021. Slugs tended to occur in the old poplars (*Populus nigra* var. *italica*). Live animals or their traces were found on the 71 of 320 poplars and on 17 trees of other species in the studied area. Slugs often go up to at least 12 m above the ground on these poplars and feed on the lichens there. Animals breed and spend the daytime inside at least some of these trees under the bark. Slugs were wintering in some of these trees in 2020-2021 and probably also at least in 2019-2020, while previously *L. flavus* was reported from Eastern Europe only in connection to cellars, basements and greenhouses. Apparently these slugs are occupying additional habitats in response to climate change and their occurrence in the trees may become common in Eastern Europe with the warming of climate. The colouration of *L. flavus* and closely related *L. maculatus* is discussed. Various differences of the colouration were suggested in the literature to distinguish the two species, most notably the central light stripe on the back of *L. flavus*, but this character is absent in most of the studied specimens and, therefore, the overall colouration overlaps in the studied populations of the two species.

Встречаемость инвазивного слизня *Limacus flavus* (Stylommatophora: Limacidae) на деревьях урбанизированного ландшафта г. Киев (Украина), с замечаниями о его окраске

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РЕЗЮМЕ. Мы изучали значение деревьев для инвазивного средиземноморского подвального слизня, *Limacus flavus*, в одном квартале жилого района в городе Киев (Центральная Украина) с весны 2020 года по весну 2021 года. Обычно слизни встречаются на старых тополях (*Populus nigra* var. *italica*). Живые особи или их следы были обнаружены на 71 из 320 тополей и на 17 других деревьях исследуемой территории. Слизни часто поднимаются по тополям на высоту не менее 12 м над землей и питаются там лишайниками. Животные размножаются и проводят дневное время внутри по крайней мере некоторых из этих деревьев под корой. Слизни зимовали в некоторых из этих деревьев в 2020-2021 и, видимо, по меньшей мере, также в 2019-2020 годах, в то время как ранее из Восточной Европы о *L. flavus* сообщалось только в связи с погребами, подвалами и теплицами. Судя по всему, эти слизни занимают дополнительные места обитания в ответ на изменение климата и их появление на деревьях может стать обычным явлением в Восточной Европе вместе с последующим потеплением. Обсуждается окраска *L. flavus* и близкородственного ему *L. maculatus*. В литературе был предложен ряд отличий в окраске для различения двух видов, в особенности центральная светлая полоса на спине *L. flavus,* но этот признак отсутствует у большинства изученных экземпляров, и, следовательно, общий окрас совпадает в изученных популяциях двух видов.

Introduction

Biological invasions are one of the most significant environmental issues of the 21st century and are known to have major negative consequences for both human enterprise and ecological systems [Pimentel *et al.*, 2000]. Many species of terrestrial molluscs are spreading far from their natural ranges, often damaging agriculture as pests and sometimes also causing decline in local faunas [Cameron, 2016].

In Eastern Europe about 40 species of terrestrial molluscs are known to expand their natural ranges, which in nearly all cases is happening in two directions: from the south to the north; and from the west to the east [Sverlova *et al.*, 2006; Son, 2010; Balashov, 2016]. Some of these species are very significant pests [Balashov *et al*., 2018a] and some apparently causing decline in the populations of native molluscs in protected areas [Balashov *et al*., 2018b]. Several

FIG. 1. Area of the study in Akademmistechko, Kyiv. Yellow dots – poplars with slugs; green dots – poplars with no signs of slugs; blue dots – other trees with slugs; *a-f* – groups of trees where many slugs were regularly observed throughout the period of study.

РИС. 1. Территория исследования в Академгородке, Киев. Желтые точки – тополя с обнаруженными на них слизнями или их следами; зеленые – тополя без признаков наличия слизней; голубые точки – другие деревья с обнаруженными на них слизнями или их следами; *a-f* – группы деревьев, на которых регулярно обнаруживались слизни на протяжении всего периода исследования.

species of molluscs has synanthropically invaded Eastern Europe a long time ago, in some cases during the pre-industrial times [Korábek *et al*., 2018]. But most of these species have started or intensified extension of their ranges during recent decades due to the change of climate [Sverlova *et al.*, 2006; Son, 2010; Balashov, 2016; Balashov *et al*., 2018a, c].

Both species of "cellar slugs", *Limacus flavus* (Linnaeus, 1758) and *Limacus maculatus* (Kaleniczenko, 1851), are extending their ranges in Eastern Europe [Likharev, Wiktor, 1980; Sverlova *et al.*, 2006; Balashov, Sverlova, 2007; Sysoev, Schileyko, 2009; Chernyshova *et al*., 2010; Son, 2010; Balashov, Gural-Svrlova, 2012; Balashov, 2016; Schikov, 2016; Ostrovsky, 2017]. The native range of *L. flavus* is considered to be somewhere in the Mediterranean region. But its borders are uncertain, as this species is among the most successful invasive molluscs. Starting at least from the middle of the 20th century it has spread nearly worldwide, including all continents except Antarctica and many islands, being associated mostly with cellars and basements [Chichester, Getz, 1969; Gittenberger, 1980; Likharev, Wiktor, 1980; Kerney *et al.*, 1983; Wiktor, 1983, 2001; Smith, 1992; de Winter, 1997; Barker, 1999; Sysoev, Schileyko, 2009; Herbert, 2010; Welter-Schultes, 2012; Rowson *et al.*, 2014a; Araya, 2015; Neiber, 2017; Schikov, 2017; etc]. The second species of the genus, *L. maculatus*, is considered to be native in Crimea, Caucasus [Likharev, Wiktor, 1980; Sysoev, Schileyko, 2009; Balashov, 2016] and maybe Anatolia and the eastern Balkans [Wiktor, Norris, 1982], as well as Donetsk Upland in Eastern Ukraine [Balashov, 2013]. But, as with *L. flavus*, the borders of the native distribution are uncertain due to introductions, especially considering that in addition to the cellars and basements *L. maculatus* often occurs in open-air habitats outside its native range. As an invasive species *L. maculatus* was common in Ireland since the 19th century and in Britain more recently [Wiktor, Norris, 1982; Rowson *et al*., 2014a], probably was present in France in the 19th century [Wiktor, Norris, 1982], has been recorded in Saint-Petersburg and its vicinities (northern part of European Russia) [Likharev, Wiktor, 1980; Sysoev, Schileyko, 2009], was recently found in Germany [Kobialka, Siedenschnur, 2017; Eta, Hausdorf, 2019], and also in Central and Northern Ukraine [Balashov, Sverlova, 2007; Chernyshova *et al*., 2010; Balashov, Gural-Sverlova, 2012; Balashov,

FIG. 2. Studied poplars with slugs in Akademmistechko, Kyiv. *a* – poplars in "group *a*" (see Fig. 1); *b* – traces of slugs on a poplar (same place).

РИС. 2. Изученные тополя со слизнями в Академгородке, Киев. *a* – тополя "группы *a*" (см. Рис. 1); *b* – следы слизней на тополе (там же).

2016]. But the actual non-indigenous distribution of this species may be largely unrecorded due to its similarity with better-known *L. flavus*.

The published records of *L. flavus* from Eastern Europe are sparse and scattered. By the 1980 it was recorded from Moscow, Saratov, Rostov-on-Don, Odessa and Yalta cities [Likharev, Wiktor, 1980]. In 2005 it was found on Zmiinyi Island (Black Sea) [Balashov, Sverlova, 2007]. In 2010 *L. flavus* was reported from the three villages of Zhytomyr region and by the one village each of Crimea, Rivne, Khmelnytskyi, Kyiv and Zaporizhzhia regions [Chernyshova *et al*., 2010]. Later it was also recorded from the cities of Kyiv [Balashov, 2016], Tver [Schikov, 2016] and Gomel [Ostrovsky, 2017]. In most of these cases it was specifically pointed out that *L. flavus* occurs only in cellars, basements or greenhouses. On few other instances it was found in gardens or other sites close to potential cellars or basements, so it was possible to assume that slugs were probably travelling from underground habitats in which they mainly lived. But until now, all published data on *L. flavus* from Eastern Europe lacked detailed observations.

As quarantine has suspended most activities in the 2020 season, we relocated our field studies to much closer areas than usual and collected the data presented here during daily walks around our home. We noticed that *L. flavus*, normally associated with basements, is often occurs on old poplars, which was evident from numerous traces of mucus. Therefore, the goal of this study was to investigate the importance of the trees to *L. flavus* in Kyiv city.

Material and methods

Observations took place from early May of 2020 to late May of 2021 within one block of the residential area in Kyiv city. It is located in the Akademmsitechko neighborhood of Sviatoshyn District, between the Dobrokhotova Str., Semashka Str. and Vernadsky Blvd., around 50°27'50"N, 30°21'58"E (Fig. 1). It's a flat plot with an altitude of about 175 m. The area of the block is about 1.6 km^2 and its largest sides are 550 m and 300 m. It comprises 24 residential buildings (18 of five-storey and 6 of nine-storey) and several of two- and one-storey buildings of kindergarten, utilities services and stores. This infrastructure was built by 1962 in an area previously dominated by pine-oak forest. At about the same time numerous trees of horse chestnut (*Aesculus hippocastanum*) and Lombardy poplar (*Populus nigra* var. *italica*; = var. *pyramidalis*; Fig. 2) were planted along the streets and pathways. Most of the poplars are currently 20-25 m tall. Between the buildings and in the public gardens numerous fruit trees are growing, mostly apple (*Malus domestica*), less frequently European

FIG. 3. Slugs of *Limacus flavus* from trees of the studied area in Akademmistechko, Kyiv.

РИС. 3. Слизни *Limacus flavus* с деревьев территории исследования в Академгородке, Киев.

pear (*Pyrus communis*) and sour cherry (*Prunus cerasus*). Also various other species of trees and shrubs were planted randomly across the block in less significant numbers. All residential buildings have basements that are only used for the purposes of utilities. The basements are rarely visited by the workers and are kept dark in rather bad conditions, at least in some of them there are proliferating populations of *Blatta orientalis* (Linnaeus, 1758), *Culex* cf. *pipiens* Linnaeus, 1758, *Scutigera coleoptrata* (Linnaeus, 1758) etc. (no specific studies were done to investigate this biodiversity, these organisms were seen outside on numerous occasions and, apparently, came from the basements). A few private cellars are present under some yards. The block is surrounded by busy road traffic, while inside it only vehicle parking takes place.

All poplar trees of the studied area were mapped, while other trees were only mapped if slugs or their traces (dried mucus) were found on them (see Fig. 1). The traces of slugs (see Fig. 2, *b*) were observed and mapped during multiple walks in the daylight, while live slugs (Fig. 3) were observed and collected during several excursions in the early morning before sunrise, and on a few occasions during the nights.

Material was collected, handled and identified using common methods of work with terrestrial slugs [Likharev, Wiktor, 1980; Kerney *et al*., 1983; Rowson *et al*., 2014a; Balashov, 2016] and is kept in the Collection of terrestrial molluscs of the I.I. Schmalhausen Institute of Zoology (Kyiv, Ukraine; SIZK, equal to IZAN).

Eleven specimens of *L. flavus* from the four different groups of poplars ("*a*", "*b*", "*c*" and "*e*" on Fig. 1; SIZK GT 7191-7194) were dissected and identified by the structure of the genitalia (Fig. 4). One *L. flavus* collected in the middle of the studied area (50°27'46.59"N, 30°21'58.13"E; north of "*f*" on Fig. 1; SIZK GT 952) in July of 2011 was also dissected, as well as three slugs collected in August 2010 from the adjacent city block (70 m from the studied block, 50°27'44.69"N, 30°21'44.46"E; SIZK GT 1706). There are no other anatomically confirmed records of *L. flavus* from Kyiv except these 15 slugs from Akademmistechko.

Some of our observations with photographs of the slugs and other organisms from the studied area and surroundings that are discussed below are available on iNaturalist (www.inaturalist.org). We have created a "Place" with the borders of the studied

area where all observations are easily accessible: "Dobrokhotova 2-16". Observations of *L. flavus* from the studied area on iNaturalist have the numbers: 47063892, 47362586, 61594261, 61603958, 61631204, 61638609.

Results

Live slugs or their traces were found on 71 of 320 poplars in the studied area (see Fig. 1). Other than poplars, traces of slugs were found on 11 fruit trees, 5 horse chestnuts and one black locust (*Robinia pseudoacacia*). About 210 live slugs were observed on all occasions before sunrise over the whole study period, some of them probably were counted several times in different days, as only a small proportion of the slugs was collected. Most of the slugs were seen on the tree trunks, some also on the surrounding ground. Up to 14 slugs were seen on the same tree at the same time (including small juveniles). On the groups of poplars that are outlined in yellow on the map (see Fig. 1) it was possible to find live slugs at every attempt before sunrise if trees were wet, as well as traces were present on these poplars throughout the whole period of study till November. On other trees the traces were seen only in some periods or in small amounts, and on some of these no slugs were seen at all. During May 2020 the number of trees with traces was lower. Some trees where no traces were seen during the summer became covered with them by October. On other trees where traces were seen during the spring or summer there were no traces by October. Traces has disappeared by early December with temperature below 0°C. It is unclear how long the traces of the dried mucus can remain, but clearly more than few days. Both adult slugs and juveniles (see Fig. 3) of different sizes were seen during almost the whole period of study from May to November. Some slugs were active during the observations on November 7th and 12th at a temperature of 4-9°C. Only several specimens were seen during these last observations, mainly juveniles. No slugs were found during winter. In 2021 the traces of slugs were observed starting from March 26, after first warming with constant temperatures above 10°C, but first only on some trees of groups "*a*" and "*b*" (see Fig. 1). By the end of May 2021 slugs or their traces were seen on about third part of the same trees where they were observed during 2020, often not the same trees that were occupied by May 2020. Therefore, slugs were wintering in some trees during 2020-2021, but clearly not in all of the trees where they were seen during 2020. It means that many slugs probably died out during a relatively cold winter (with air temperatures below -20°C in some days) or perhaps largely migrated to underground habitats before winter.

During the studied period no slugs were seen in the daytime even after the rain in rainy weeks. Numerous traces were also seen regularly on the four particular sewer hatches (out of many) in four different parts of the city block next to the trees with slugs ("*b*", "*d*", "*e*" and "*f*" on Fig. 1). In one place the traces were seen on a ventilation stack of a cellar next to the poplars with slugs ("*c*" on Fig. 1). The interiors of basements, cellars and sewers were not investigated.

It was directly observed that slugs were hiding inside crevices in the trunk and bark of the poplars in the morning, so there could be no doubt that they are spending the daytime in at least some of these trees on some days. As these trees are relatively large and old, there are numerous cavities of various sizes inside many of them.

Slugs were often climbing up to at least 12 m above the ground on the studied poplars, as seen from their traces. We observed several trees with 12x binoculars and compared the height with nearby buildings. The dried mucus shines in sunlight (see Fig. 2, *b*), so it can be spotted even from several meters away.

On the poplars the slugs are feeding on lichens. Poplars on which slugs or slug traces were not seen appeared to have more lichen cover. The lichens present on the studied poplars were identified as *Xanthoria parietina*, *Phaeophyscia orbicularis*, *Physcia adscendens*, *Physcia tenella* and *Myriolecis hagenii* by Valerii Darmostuk on iNaturalist, photos available there.

Hedgehogs, *Erinaceus roumanicus* Barrett-Hamilton, 1900, are common in the studied area and were regularly seen next to the trees with slugs during the nights and early mornings. They may eat *L. flavus* there, but we did not see this directly.

Poplars with traces of slugs are also common in two adjacent blocks of the residential area, to the south and to the west of the studied block. Rows of poplars grow also along the center of Vernadsky Blvd., on the green avenue between the two traffic ways of the boulevard (north and northwest from the studied block, these rows of poplars are visible on Fig. 1). Traces of slugs were specifically searched for on these poplars, but none were found.

We have noticed no traces of slugs on the studied trees before the spring of 2020, but we are not entirely sure whether they were absent or we overlooked them (first author is a resident of this block since 1985).

Before the period of this study, in July 2011, one live slug of *L. flavus* was found in the studied area on the footpath next to the living building in daytime during rain. It was several meters from the place where it could enter the basement of a nearby building. There are no poplars or entrances to the sewers around this place. On another occasion, in August 2010, about 10 *L. flavus* were found next to the footpath in the adjacent block of the city in the daytime. These slugs were found on paper, so it looks

like they were collected in the paper and deliberately thrown away there by someone, perhaps from one of the cellars or basements nearby.

Other than *L. flavus* only one species of slugs has been recorded within the studied block of the city, on a few rare occasions – invasive *Deroceras caucasicum* (Simroth, 1901). Few adult specimens were collected and dissected in previous years. During the period of study a few juveniles, probably of *D. caucasicum*, were seen. Snail *Trochulus hispidus* (Linnaeus, 1758) was found in few sites of the studied block in the yards. Also during the period of study (and never before) few adult specimens of *Helix pomatia* Linnaeus, 1758 were observed in different parts of this area, but probably these snails were occasionally delivered (e.g. by children) from the numerous populations on the outskirts of the city (in about 1.5-2 km from the studied area). No other molluscs were seen in the studied area.

The closest locations from which *Limacus maculatus* has been recorded are 1.5-2 km north from the studied area. One slug was found recently in a semi-natural pine-oak forest along a highway (29.05.2020; 50°28’53.1”N, 30°21’46.5”E); photos of the slug and its genitals are available in the observation 47718268 on iNaturalist. Several slugs of *L. maculatus* were also found nearby among the private cottages in June 2014 (collected by Prof. L.S. Balashov, around 50°28’32”N, 30°21’25”E; genitalia checked, two slugs with unusual colouration from this locality are shown in Table IV in Balashov, 2016). In Kyiv *L. maculatus* was also recorded from the park landscapes of the A.V. Fomin Botanical Garden (2011-2020) and of the Syretsky Park (2008). We have also seen *Limacus* slugs on many other occasions across the city during the last decade (and likely their traces in even more locations), but have not collected these slugs to identify them by the genitalia. The first record of *L. maculatus* from Kyiv Region was in 2006 from Vasylkiv town [Balashov, Sverlova, 2007], before that there were no records of *Limacus* from Ukraine outside its southern regions.

On the outskirts of the city 1.5-2 km west and north from the studied block, in the area where *L. maculatus* was recorded and nearby, several other species of slugs are occurring: *Limax maximus* Linnaeus, 1758 (Limacidae), *Deroceras reticulatum* (Müller, 1774), *D. caucasicum*, *Krynickillus melanocephalus* Kaleniczenko, 1851 (Agriolimacidae), *Arion circumscriptus* Johnston, 1828 (rare), *Arion fasciatus* (Nilsson, 1823) and *Arion fuscus* (Müller, 1774) [=*A. subfuscus* auct.] (Arionidae). Apparently, the blocks of residential area with the high-rise buildings are not suitable for most of these slugs in Kyiv.

As of 2020 there are no *Arion vulgaris* Moquin-Tandon, 1855 [=*A. lusitanicus* auct.] in this part of the city so far. This important pest became very abundant in some other parts of Kyiv during recent years [Balashov *et al*., 2018a] and is expected to spread more widely across the city in the near future.

Discussion

Our data suggests that *L. flavus* is living, feeding and breeding within some of the studied poplars. Apparently, the adult slugs can travel on large distances during one night, perhaps up to several tens of meters. But small juveniles (see on Fig. 3) are obviously much less mobile and it seems impossible for them to cross the pathways or to travel in the sewers or basements regularly. Therefore, the presence of numerous juveniles is indicative of breeding inside the trees. We recorded many juvenile slugs of all sizes during May on some poplars, most notably on the group of 10 poplars (“*a*” on Fig. 1) where there are no adjacent entrances to sewers or basements. Slugs have to cross the wide pathways (1.5-2.5 m) with the bituminous or tile surfaces to get there. Moreover, the traces of slugs were never seen on the sewer hatch nearest to this place. It suggests that slugs or their eggs probably were wintering at least in some of these poplars during the 2019-2020 and this is even more evident for winter of 2020-2021, as on some trees the traces were seen from the first warming in March 2021.

There are entrances to the sewers right next to the trees in 4 of the 6 groups of poplars where we saw many slugs regularly (“*b*”, “*d*”, “*e*” and “*f*” on Fig. 1) and in one more case there is a cellar (“*c*” on Fig. 1). Considering the numerous traces there is no doubt that adult slugs are often travelling from underground to the surface in these five sites. It suggests that slugs are probably spreading through the sewers and from there colonizing some sites on the surface. As we did not study the sewers it may also be possible that slugs disperse on the ground and use sewers as secondary refuges, or they may disperse in both ways. On most of the sewer hatches of the studied area we saw no traces of slugs, but perhaps this is because there are no slits where slugs could easily travel to the surface.

The slugs of Kyiv were repeatedly studied for over 150 years [Jelski, 1863; Panotshini, 1929; Baidashnikov, 1992; Tappert *et al*., 2001; Sverlova *et al*., 2006]. But there are no any records of *Limacus* species before the 2000s. It is unlikely that these large unique-looking animals were overlooked or misidentified for such a long time. Therefore, most probably, their expansion there has begun, or at least much intensified, during the last two decades in the response to climate change. The slugs of *L. flavus* first occupied closed constructions and now, apparently, they are on the beginning of the next stage with a tendency to occupy also the open-air habitats as a result of the further changes in climate.

It is notable that occurrence of the *Limacus* spe-

cies across Eastern Europe appears to be much underestimated in the existing literature. As is seen from the numerous photos on iNaturalist and other online databases, these slugs are currently rather common at least in many settlements across Central, Eastern and Southern Ukraine, Belarus and central part of European Russia. Absence of such records before the 2010s also supports a hypothesis that *Limacus* species have much intensified their spread in Eastern Europe recently in response to climate change. Most of the *Limacus* slugs from Eastern Europe on iNaturalist and other online databases are identified as *L. flavus*, but probably many, or even most of these photos are actually representing *L. maculatus*. It seems that both species of *Limacus* are currently widespread and common in the settlements across at least the southern half of Eastern Europe. With further climate change these species will probably become even more widespread and common.

Obviously not all trees are suitable for *L. flavus*. One of the main reasons why slugs have occupied the studied poplars is their age and size, these trees are large and with many cavities. The poplars with smaller trunks are clearly less occupied by *L. flavus*. But slugs are almost absent from the horse chestnuts that were planted at the same time in similar numbers across the studied block. Some of these trees have large trunks with many cavities, often covered with lichens, though they are always much shorter than poplars in the studied area. Perhaps the reason why poplars are suitable for the slugs is the deep structure of their bark with large cracks (see Fig. 3), while in the horse chestnuts the bark is relatively smoother. Such structure of the poplars' bark is keeping more moisture on its surface and allows slugs to hide in the cracks.

Presence of *L. flavus* in the urban landscape seems to be harmless or even beneficial. This slug is not considered to be a significant pest [Rowson *et al*., 2014a]. It feeds mainly on fungi and algae, including lichens. Therefore, it may not significantly damage the trees or other living plants. The slugs eat lichens and other fungi that could be harmful for the trees, so presence of these slugs is probably rather good for the trees and perhaps could even prolong their lifespan. *Limacus flavus* may be consumed by hedgehogs and some birds and provide a food source [Barker, 2004]. In the studied area the slugs are probably serving as food for hedgehogs (*Erinaceus roumanicus*). Perhaps the juvenile slugs also could be occasionally consumed by *Turdus merula* Linnaeus, 1758 (common blackbird), *Sturnus vulgaris* Linnaeus, 1758 (common starling) and *Dendrocopos major* (Linnaeus, 1758) (great spotted woodpecker) that occur in the studied block (latter is less common, was observed several times during the studied period). Therefore, *L. flavus* probably could support a diversification of the biodiversity in the urban landscape. A potential threat is if *L. flavus* will be able to live in natural habitats of Eastern Europe with further climate change. In such case the slugs could invade natural habitats from the urban landscapes and it could threaten native slugs and lichens.

Revealing many of the details in the present study only became possible because there are no molluscs other than *L. flavus* that could have left such traces in the studied area. A second slug that was found here, *Deroceras caucasicum*, as well as a snail *Trochulus hispidus*, are too small, especially during the period of study, most of the slug trails are much wider than bodies of these molluscs. If *Helix pomatia* was responsible for the part of these traces, we would clearly find much more than two snails. Moreover, the traces of *H. pomatia* appear to be much less prominent than those of large slugs. Therefore, there is no doubt that the numerous *Limacus* slugs seen by us during the mornings and nights are responsible for all or nearly all of the traces recorded during the daytime. But a second species of the genus, *L. maculatus*, is also present in this part of the city with the closest anatomically confirmed location in about 1.5 km from the studied area. Together with the fact that the colouration of the studied slugs (see below) does not fully correspond to the description of *L. flavus* in the best-known recent guide on the slugs of Europe [Rowson *et al*., 2014a], it makes it necessary to address our identification of the *Limacus* species in detail.

We dissected 11 slugs from 4 different groups of trees during the period of study, as well as 4 other slugs from this area and surroundings collected in previous years. The genitalia of these 15 specimens shows low variability. In all specimens the duct of the bursa copulatrix is attached to the oviduct, not to the atrium, and the penis is relatively long and curved few times (see Fig. 4). In all studied specimens of *L. maculatus* from Kyiv Region (also about 15, in 2006-2020) the duct of bursa copulatrix is attached to the atrium or in some cases rather to the base of the penis, and the penis is short, with only one curve. The place where the duct of the bursa copulatrix is attached is the main distinctive character by which the two species of *Limacus* are divided, while the length of the penis is a secondary character that also works in most of the cases [Giusti, 1973; Likharev, Wiktor, 1980; Wiktor, Norris, 1982; Wiktor, 1983, 2001]. There were no transitional forms or doubtful specimens, therefore dissecting more than 11 specimens for the present study appeared to be unnecessary. The colouration of the slugs from the studied area was also not very variable. All the slugs looked more or less like ones on the photos (see Fig. 3), which is a common colouration for the most specimens of both *Limacus* species seen by us (including numerous *L. maculatus* from the Crimean Mountains, their "terra typica"). At the same time *L. maculatus*

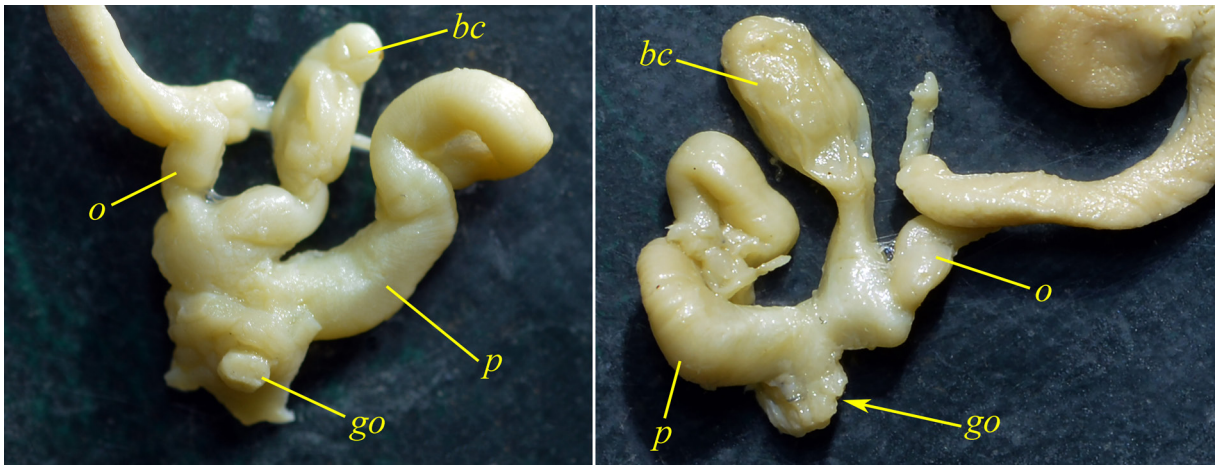

FIG. 4. Genitalia of *Limacus flavus* from trees of the studied area in Akademmistechko, Kyiv (two specimens). *bc* – bursa copulatrix; *go* – genital opening; *o* – oviduct; *p* – penis.

РИС. 4. Гениталии *Limacus flavus* с деревьев территории исследования в Академгородке, Киев (два экземпляра). *bc* – яйцеприемник; *go* – половое отверстие; *o* – яйцевод; *p* – пенис.

from Kyiv, especially from the studied part of the city, show greater variability in colouration. Many slugs were much more yellowish than usual *Limacus* colouration, and some specimens even have large dark stripes along the sides of the body similar to those in some specimens of *Limax maximus* (see Table IV in Balashov, 2016). We have seen no slugs with such unusual colouration in the area of study, which also suggests absence of *L. maculatus* in the studied city block.

It is notable that the light stripe along the back was present only in some of the studied slugs, mostly in the juveniles, and did not extend the whole length of the back. But this character was reported as a distinctive one for *L. flavus* from the British and Irish colonies of this species [Rowson *et al*., 2014a]. We can assume that this character may be really useful to distinguish the two *Limacus* species in Britain and Ireland and, in such case, it is fully legitimate for the guide on the slugs of these countries. But, evidently, it doesn't work for the colonies in Kyiv Region and, therefore, cannot be used to distinguish these two species across their whole ranges, as with other supposed differences in the colouration that were proposed in the past [Likharev, Wiktor, 1980; Wiktor, Norris, 1982; Evans, 1982, 1983]. In Britain and Ireland both *Limacus* species are invasive and their colonies could originate from the very limited number of specimens, or even a single slug for each species, as these animals are capable of self-fertilization [Evans, 1983]. Therefore, the genetic diversity of these colonies is likely to be relatively low and slugs from these colonies are expected to be less variable than their species overall. Also the slugs from the anthropogenic environment could potentially possess some characters that would be wiped out in their native ranges by natural selection, which perhaps could explain an unusual colouration of the *L. maculatus* from Kyiv mentioned above [Table IV in Balashov, 2016]. Consequently, dissection is always required for the precise identification of the two *Limacus* species, except maybe some regions where their external variability is well-studied or only one species is confirmed to occur so far. Though, considering the available genetic data from the two species [Rowson *et al*., 2014b], the real situation is probably even more complicated. Possible hybrids of the two *Limacus* species were reported, as well as specimens that genetically are *L. flavus*, but anatomically resemble *L. maculatus* [Rowson *et al*., 2014b]. It is hard to judge this data without seeing the actual morphological characters of the involved slugs. These results are also based mainly on the material from the invasive colonies of the two species, while the starting point for such studies should be in their native populations, preferably with the designations of the neotypes for both species, or at least *L. flavus* which lacks a type locality. The current state of knowledge only indicates that a larger molecular study on the phylogeny of the *Limacus* species is required. Extensive material from the natural habitats of the Crimean Mountains, Caucasus, Anatolia, the Balkans and probably western Mediterranean regions should be involved in such a study. Until then the classical morphological concept of the two *Limacus* species should be used, in which the place of attachment of the duct of the bursa copulatrix is the main distinctive character [Wiktor, Norris, 1982].

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