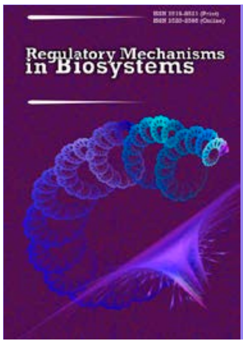

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Possibility of identifying plant components of the diet of *Harpalus rufipes* (Coleoptera, Carabidae) by visual evaluation

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Harpalus rufipes (De Geer, 1774) is a trans-palearctic, polyzonal, habitat generalist species, which is usually the most numerous ground beetle species in agricultural ecosystems and forest plantations. In laboratory conditions, 50 *H. rufipes* imagoes were placed in separate containers, each individual being fed over several days with seeds of a single species of plant, the total number of plant species being ten. Then the content of the beetles' intestine were analyzed using Lugol's iodine stain for visualizing starch granules. Native agents of ground seeds of plants and also of seeds treated by a fermentative agent from a mammalian pancreas were used for control. Granules of starch from seeds of *Triticum aestivum* L., *Hordeum vulgare* L. and *Secale cereale* L. were only insignificantly broken down by enzymes in the intestines of *H. rufipes*. The starch granules of *Avena sativa* L., *Panicum miliaceum* L., *Sorghum drummondii* (Steud.) Millsp. and Chase, *Fagopyrum esculentum* Moench and *Sinapis arvensis* L. were also insignificantly affected in the beetles' intestine compared to the agent affected by enzymes of vertebrate animals. Starch granules of *Beta vulgaris* L. seeds affected by the enzymes became deformed and fragmented. Sometimes only their fragments remained. Seeds with a high content of fats such as seeds of *Juglans regia* L. were digested poorly in the intestine of *H. rufipes* (drops of fat could be seen surrounding certain food particles, which obstructed their digestion). The results of microscopic study of the intestinal content of mixed phytophage ground beetles of agricultural environments will help in identifying mechanisms of regulation of trophic chains by polyphage species, and will help advance the study of gregarine infection rates among ground beetles.

Keywords: intestinal content; ground beetles; digestion; starch; mixed phytophages

Introduction

Identifying the dietary components of mixed phytophage ground beetles is an interesting and promising area of ecological research. Ground beetles which feed on plant seeds can consume both cultivated species of plants, therefore causing damage to agriculture, and weed seeds, which is beneficial to agriculture (Honek et al., 2003; Fawki and Toft, 2005; Klimeš and Saska, 2010). Studying the diet of different species of ground beetles which feed on plants in the conditions of natural ecosystem and agricultural landscapes is interesting both from a theoretical (evaluating the structure of trophic chains, identifying the mechanisms of regulation of phytocoenosis structures, etc.), and from a practical perspective (identifying mechanisms of limiting the population of the most harmful species of weed, qualitative evaluation and quantification of seed stock of weeds in agrocoenoses, etc.).

One such mixed phytophage-species is *Harpalus rufipes* (De Geer, 1774), a trans-palearctic polyzonal species inhabiting fields, typically the most numerous species of among the ground beetle in agricultural ecosystems (Kryzhanovskij et al., 1995; Hurka, 1996; Freude et al., 2004; Kataev and Liang, 2015). The biology of *H. rufipes* in natural conditions has been studied in some detail (Lindroth, 1986; Midtgaard, 1999; Porhajašova et al., 2009; Harrison and Gallandt, 2012). *H. rufipes* consumes agricultural pests, and also causes damage to cereal cultures. *H. rufipes* is usually found in cultivated lands, pastures, in gardens, in polluted areas, in ruderal communities (Lindroth, 1986; Davies, 1953). In most habitats, *H. rufipes* lives together with 3–7 species of the *Harpalus* genus, generally being numerically dominant and attaining the maximum size among the entire group of ground beetles with a mixed (vegetative and animal) diet (Thomas et al., 2001). The life-cycle of *H. rufipes* varies (Matalin, 2007): in the north of its range, a generation develops over two years, in the south part – during one year. Imagoes are seen from late March – April to late September – November. Oviposition takes place from early Mach to July. In the two year development cycle, larvae and imagoes hibernate (Lindroth, 1986).

In spring and in early summer, *H. rufipes* migrates over quite large distances, moving 10–15 m a day. H. rufipes can make significant migrations by land and by air, with clusters of dozens and hundreds of individuals per square meter in areas with optimum hygrothermal regimes and concentrations of trophic objects (vegetative and animal). In the areas, where individuals concentrate, they can significantly affect plantings of agricultural crops (Lindroth, 1986; Currie et al., 1996). Studying the trophic chains between this species and seeds of different plant species is valuable agriculturally and scientifically for developing the methods of general calculation of the population of any species in an agrocenosis. Selectively consuming seeds, the beetle can cause a much stronger effect upon the structure of a phytocoenosis than it would by consuming the vegetative parts of plants (Honek et al., 2003; Saska et al., 2010). On the other hand, it is possible that individual preferences exist among different individuals within populations (Korolev and Brygadyrenko, 2014), therefore the availability of certain types of food is interesting for studying intra-populational trophic and morphological polymerphism of a *H. rufipes* population (Brygadyrenko and Reshetniak, 2014a).

In the conditions of steppe zones, beetles are common in forest plantations and natural forests of different types Brygadyrenko (2014, 2015). Consuming seeds of different plant species and invertebrates of different species (Monzo et al., 2011; Reshetniak and Brygadyrenko, 2013), they are able to survive through unfavorable periods of droughts, when they concentrate on the edges of grain crop fields and limit the seed yield both for cultivated cereals and weeds in the fields (Faly and Brygadyrenko, 2014; Faly et al., 2017). The factors which cause the imagoes of this species to migrate (including mass migrations) have been insufficiently studied (Midtgaard, 1999), but certainly apart from beetles' activity related to mating and oviposition in the most favorable conditions, the main factor is searching for a sufficient amount of seeds of optimum quality and of optimum species composition, including in agrocoenoses (Zhang et al., 1997; Hartke et al., 1998; Porhajašova et al., 2009; Bohan et al., 2011; Harrison and Gallandt, 2012). Clusters of *H. rufipes* imagoes attract a great number of predators (Thiele, 1977; Churchfield et al., 1991), which drives the movement of energy in ecosystems further up the trophic chains.

Field studies have indicated a high extent of infection of *H. rufipes* with several species of gregarines (Brygadyrenko and Reshetniak, 2016). The influence of the dietary components of different beetle individuals upon these species of protozoan parasites remains unstudied. It is another interesting and important practical research area where microscopic identification of the beetles' diet components has a significant value. Experiments on seed consumption by *H. rufipes* have been conducted for a large spectrum of agricultural crops (Hartke et al., 1998; Shearin et al., 2008; Brygadyrenko and Reshetniak, 2014b). The seeds of plants are the main source of energy for *H. rufipes*, which the beetle specifically searches for. For example, *H. rufipes* selectively eats out the seeds of strawberries (Briggs, 1965). The species provides great interest for studying the process of breakdown of starch in the intestines of polyphage insects.

Therefore, the objective of this paper is to evaluate the possibility of determining the content of the diet of *H. rufipes* using the visual characteristics of their intestinal content in laboratory conditions.

Materials and methods

H. rufipes individuals were collected using pitfall traps in August 2015 in corn fields around Dnipro (central part of Ukraine). The beetles were collected from a population with a low level of gregarine infection, and indeed not a single gregarine specimen was found during the course of the experiment. 50 *H. rufipes* individuals were used in the research. The beetles were put in separate containers (to prevent cannibalism) with no food, but with access to fresh water and were kept for two days for cleansing their intestines from undigested food remains. Then each *H. rufipes* imago was kept separately in a rectangular container (8 × 12 cm and 8 cm height) with one type of food for 5 days. Overall, the *H. rufipes* imagoes were fed with fruits and seeds of 10 species of food plants (5 beetles for each food plant species). Each beetle was offered one food type in excess quantity (Table 1).

Table 1
Brief characteristics of food substrates of *H. rufipes* beetles in the laboratory experiment

Family	Species	English name	Part of plant	Processing method
Poaceae	*Avena sativa* L.	Oat	fruits	solid
	Secale cereale L.	Rye		
	Hordeum vulgare L.	Barley		
	Sorghum drummondii (Steud.) Millsp. and Chase	Sudan grass		
	Panicum miliaceum L.	Proso millet		cleaned from shell
	Triticum aestivum L.	Bread wheat		solid
Polygonaceae	*Fagopyrum esculentum* Moench	Buckwheat		cleaned from shell
Chenopodiaceae	*Beta vulgaris* L.	Sea beet		solid
Juglandaceae	*Juglans regia* L.	Persian walnut		cleaned from shell
Brassicaceae	*Sinapis arvensis* L.	Field mustard	seeds	solid

The numbered containers were arranged randomly on the laboratory table, which was not exposed to direct sunlight. The temperature in the laboratory fluctuated from +22 °C in the night to +28 °C in the day, relative air humidity was 38–54%. The entire laboratory part of the study was completed in 5 days, from 08–13.08.2015 (in the period of maximum mobility of the beetles common in Ukraine's steppe zone).

The intestines of the beetles were removed and placed on microscopic glass in physiological solution. Then 5–6 transversal incisions by scalpel were made, extruding the content of the middle section of intestine. The elements of digested food were stained using Lugol's iodine for detailed visualization of starch granules.

For comparison of the intestinal content with standard samples, the samples of all the fruits and seeds used in the experiment were ground in a porcelain mortar, then placed on the microscopic glass in the physiological solution heated to 36 °C with fermentative preparation from the human pancreas. The preparation was also stained using Lugol's solution. The control samples of raw fruits and seeds were also ground in a porcelain mortar, and then put on a microscopic glass in physiological solution and stained in a similar way.

The studied material was photographed using a digital camera with 5 megapixel resolution. The observations were made using a microscope with ×5, ×10 magnification and ×40 planochromatic microscope objectives.

Results

No differences were found among the five specimens in each group which consumed a particular plant species, the content of their intestine being monotypic. However, significant differences were found in intestinal content of the beetles which were fed with different types of food (Fig. 1–4). Starch grains in the content of the middle section of the beetles' intestine and in the preparation treated with human digestive enzymes did not significantly differ in form or in size.

Starch granules in wheat seeds are oval, 2–5 µm in diameter (Fig. 1*a–c*). By contrast, starch granules of rye are on average larger (3–7 µm), most often round (Fig. 1*d–f*). Starch granules of barley are smaller, most often round rather than oval (Fig. 1*g–i*). Fermentative preparation breaks down granules of these cereals more intensively than the enzymes of *H. rufipes* intestines.

Starch granules of oats (Fig. 2*a–c*) are much smaller than the granules of the above-mentioned cereals (0.7–1.8 µm). Starch granules of millet (Fig. 2*d–f*) are larger (1.5–2.8 µm). Starch granules of sorghum (Fig. 2*g–i*) are the same size as starch granules of wheat – 2.0–4.5 µm. In the beetles' intestines they were not affected compared with the preparation affected the enzymes of vertebrate animals.

The starch granules of buckwheat (rhombic, 2.5–4.0 µm length, Fig. 3*a–c*) and mustard (irregular shape, 1.5–5.0 µm, Fig. 3*d–f*) remained unaffected in the intestines of *H. rufipes*. Starch granules in the seeds of beet (rhombic, 2.5–4.0 µm length, Fig. 3*g–i*) affected by enzymes of *H. rufipes* intestines became deformed and fragmented, often only small particles of them were left.

Seeds with high fat content (for example Persian walnut) were digested poorly in the intestines (Fig. 4) of *H. rufipes* beetles. Drops of fat (more than 10–15 µm in length) were visible around separate particles, which impeded their digestion.

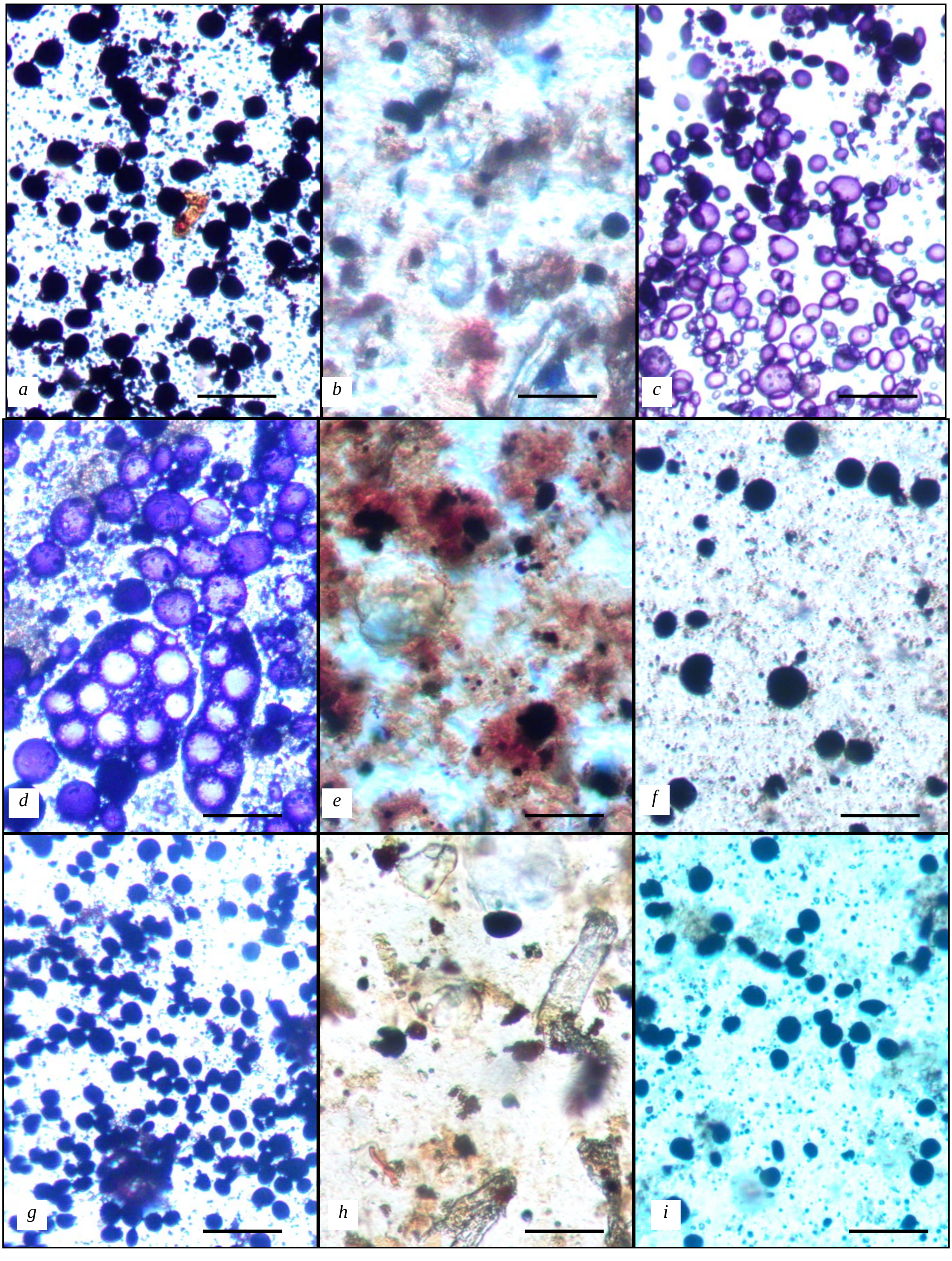

Fig. 1. Influence of fermentative preparation from the human pancreas and intestinal enzymes of *H. rufipes* upon the seeds of wheat (*a*–*c*), rye (*d*–*f*) and barley (*g*–*i*): *a*, *d*, *g* – ground seeds, *b*, *e*, *h* – ground seeds processed using fermentative preparation, *c*, *f*, *i* – seeds taken out of the middle section of intestines of *H. rufipes*; staining on all preparations was done with Lugol's solution; bar – 10 µm

Discussion

In our previous research we analysed the digestion of different plant foods by *H. rufipes* (Brygadyrenko and Reshetniak, 2014b) and found that fat – rich foods were consumed less and caused a decrease in the beetles' body mass. By using food mixtures containning several species of plant foods we found the optimum combination for food consumption and increase in the beetles' body mass – for the most efficient digestion of food. The current research is a continuation of our previous series of experiments on finding optimal conditions for keeping beetles in a laboratory, including the provision of artificial types of diet (Reshetniak and Brygadyrenko, 2013). In the photographs of the intestinal content of the beetles which are fed on the seeds of Persian walnut (Fig. 4) fat drops around food particles could be clearly distinguished, which impeded the beetles' normal digestive process.

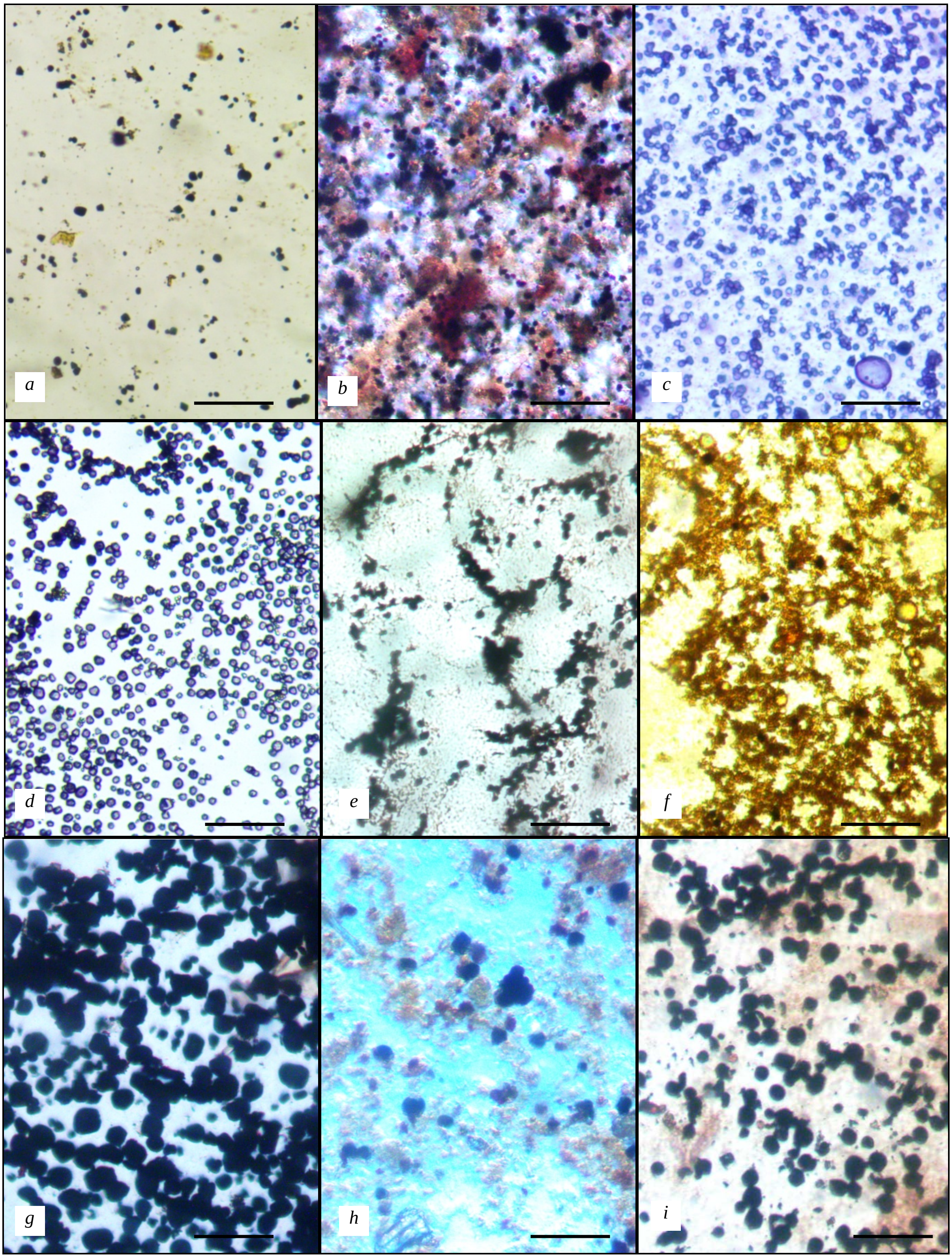

Fig. 2. Influence of fermentative preparation from the human pancreas and intestinal enzymes of *H. rufipes* upon the seeds of oat (*a*–*c*), millet (*d*–*f*) and sorghum (*g*–*i*): *a*, *d*, *g* – ground seeds, *b*, *e*, *h* – ground seeds processed using fermentative preparation, *c*, *f*, *i* – seeds taken out of the middle section of intestines of *H. rufipes*; staining on all preparations was done with Lugol's solution; bar – 10 µm

The second aspect of this research was the influence of the structure of the content of the central section of the intestine of *H. rufipes* on morphological variability of the single cell parasites which we often find in the course of our research (Reshetniak, 2015). We have found that gregarines change their morphology in different beetles (Brygadyrenko and Reshetniak, 2016). The morphology of gregarines is the main indicator in identifying their taxonomic position. However, we have found no research in the literature on the influence of the content of partially digested food on the morphological characteristics of gregarines, parasites of insects. Even though no gregarines were found in the intestines of the beetles collected, this article is the first attempt at visual assessment of the environment for gregarines in a laboratory experiment with the hosts feeding on a single type of food. *H. rufipes* is not a pure phytophage;

apart from plants, the beetles consume dozens of species of invertebrates, which impedes morphological study of the beetles' intestinal content.

Changes in the structure of starch grains occur in the rear part of the central section of the intestine of *H. rufipes*. Therefore, we found no morphometric changes in the form of starch grains.

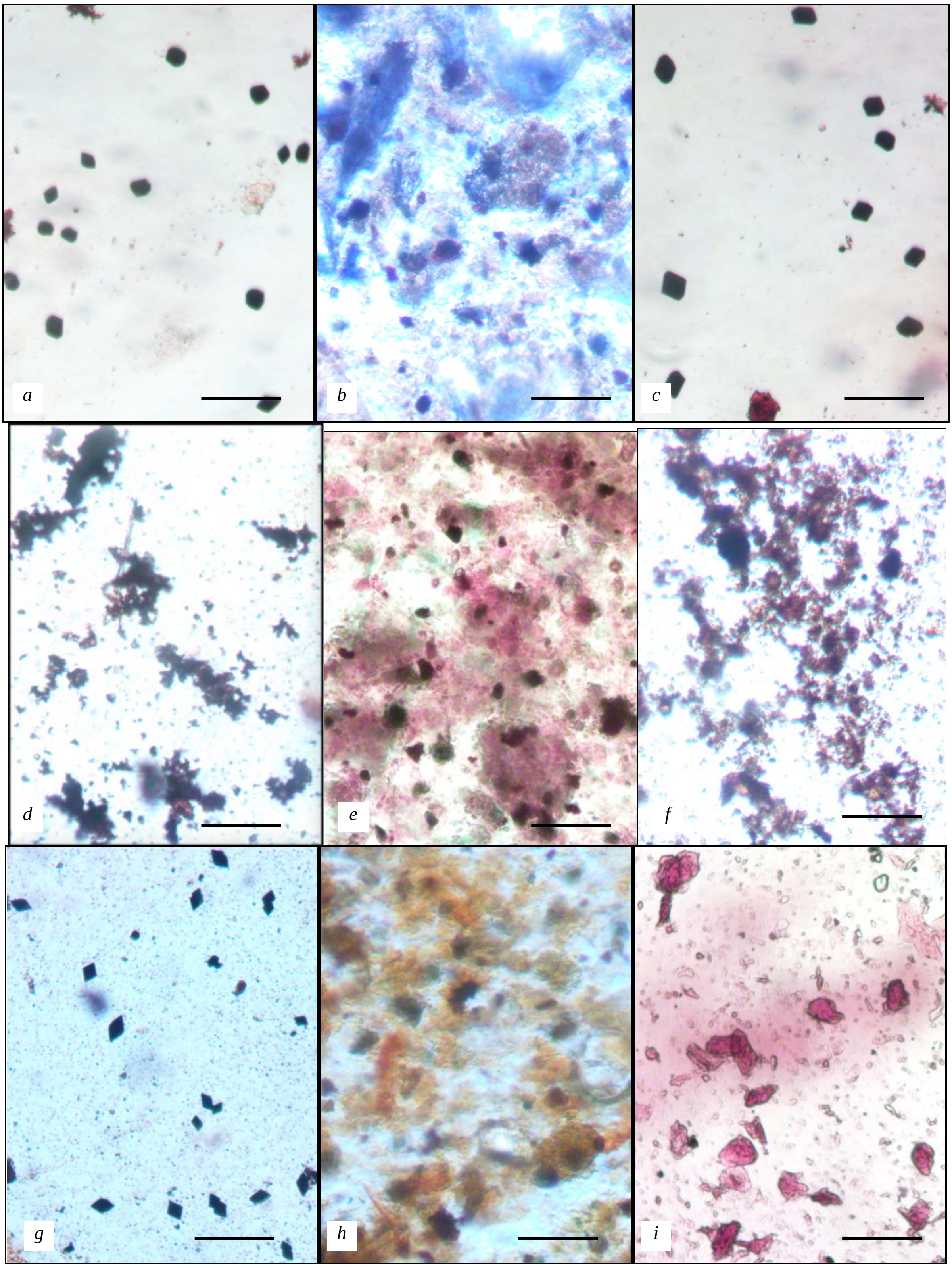

Fig. 3. Influence of fermentative preparation from the human pancreas and intestinal enzymes of *H. rufipes* upon the seeds of buckwheat (*a*–*c*), mustard (*d*–*f*) and beet (*g*–*i*): *a*, *d*, *g* – ground seeds, *b*, *e*, *h* – ground seeds processed using fermentative preparation, *c*, *f*, *i* – seeds taken out of middle section of the intestines of *H. rufipes*; staining on all preparations was done with Lugol's solution; bar – 10 µm

Identifying plant species whose traces were found on the stone tools of cavemen is only possible using the methods of identifying the peculiarities of morphometric and optical properties of starch granules. Starch is the main component of any plant seed – it can make up 70% of their dry weight (Arraiz et al., 2016). The starch from seeds of *Avena sativa* was resistant to the effect of different HCl concentrations at temperatures of 25 °C and 50 °C (Bet et al., 2016). Cleaned seeds of buckwheat also are a rich source of starch for *H. rufipes* beetles (Dziadek et al., 2016). The results we obtained indicate that using microscopic analysis of beetles' intestinal content allows the qualitative composition of their diet to be identified, which enables the most significant components to be determined and the variation of the diet for different *H. rufipes* individuals to be assessed (Brygadyrenko and Reshetniak, 2014b).

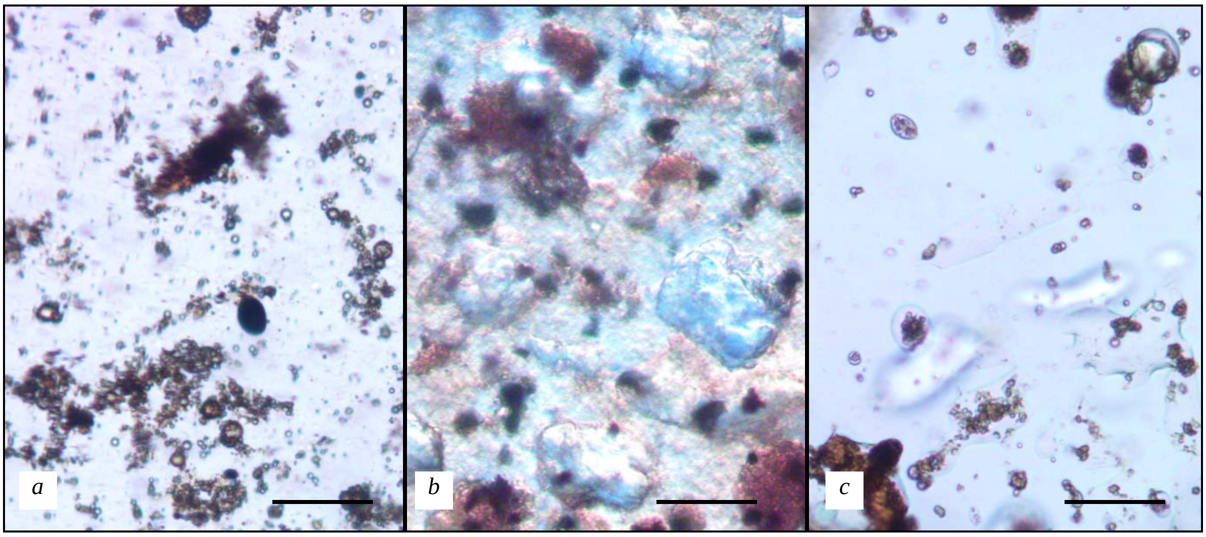

Fig. 4. Influence of fermentative preparation from the human pancreas and intestinal enzymes of *H. rufipes* upon the seeds of Persian walnut: *a* – ground seeds, *b* – ground seeds processed using fermentative preparation, *c* – seeds taken out of the middle section of intestines; staining on all preparations was done with Lugol's solution; bar – 10 µm

Conclusion

Usage of visual analysis of beetles' intestinal content is a possible additional method of analysis, which is promising for employment together with PCR-methods. Studying the trophic chains of *H. rufipes* and other species of ground beetles in particular agrocoenoses and natural ecosystems enables interesting mechanisms of regulation of trophic chains by polyphage-species to be determined. This research method will be a significant addition to parasitological methods of studying ground beetles' intestinal content when investigating their infection with gregarines, nematodes and other parasitic species.

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