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A REVIEW ON BENZANTHRONE LUMINOPHORES FOR RAPID AND HIGH-RESOLUTION IMAGING OF PARASITES BY CONFOCAL LASER SCANNING MICROSCOPY

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Abstract

Nowadays, is growing interest in investigation of parasites and their anatomic structure. Most common tools are linked with usage of luminophores and luminescent microscopy techniques. In recent years, a variety of fluorescent probes have been developed and widely used to realize the visualization of certain structures [1]. The benzanthrone compounds captivated a lot of interest as fluorescent probes for biomedical technologies because of remarkable spectral properties and negligible fluorescence in the aqueous phase [2]. Notably, the spectral characteristics of benzanthrone dyes meet the criteria for an ideal bio-imaging agent, featuring a high extinction coefficient, bright fluorescence, photo-, thermo- and chemical stability, and reduced background signal [3]. Fluorescent molecular dyes, currently used to study cell membranes, make lipid structures visible through optical techniques [4] and act as potential fluorescent probes for biomolecules [5]. There are studies in the literature where the benzanthron phosphors have confirmed their application for visualization of biological objects [6-8]. Despite high activity in this field, still it`s huge request for specific fluorescent probes for biological objects.

The confocal laser scanning microscope (CLSM) is a powerful tool for providing high-resolution optical sections. CLSM can be used to analyse images of morphological structures and to place organ or organ systems of interest in their anatomical context. Luminescence imaging techniques are increasingly utilized for exploring the structure and properties of biological objects. Laser-induced fluorescence stands out as a sensitive method, capable of detecting even a single-molecule under specific conditions [9], which makes it a powerful bioanalytical tool for life sciences [10-12]. Benzanthrone derivates are used for CLSM imaging as fluorescence probe for various biological objects because benzanthrones render the specimen fluorescent to examine the stained samples by optical sectioning [13,14]. This review provides inputs in utilisation of different benzanthrone luminophores for examination of the parasites.

Key words: parasites; luminophores; confocal laser scanning microscopy; staining protocols.

Introduction

Parasites play an important role in healthy and functioning ecosystems. They are part of food chains, biological cycles and even serve as environment indicators showing changes in various habitats [15].

Parasite species identification is crucial for life cycle description, determination of prevalence and intensity, biogeographical distribution and for interaction among species [16,17].

A simple tool used to identify species is a taxonomic key. Taxonomic keys are created based on a specific character present in the group of organisms, these characters are quantitative or qualitative. However, taxonomic keys are created based on group of organisms, which are living in a specific region at a specific time period, therefore, not always an examined organism will comply to all taxonomic keys in the list. There could be minor variations. This is the reason why taxonomic keys are challenging to describe as they would be used worldwide [18].

Modern microscopic methods such as CLSM allows to obtain high-resolution optical images. Thus, increasing our understanding of parasite's biology. The most important advantage of this method is that it allows to visualize and examine both fresh and previously fixed samples. Nowadays, CLSM is widely used for investigation of muscular arrangement [19,20], parasite's internal and external structure [21,22] or to examine general morphology of parasite's specific attachment organs such as oral suckers [14]. All these studies describe more and more species-specific characteristics; therefore, the confocal microscopy method gives more detailed information about species specific characteristics and these differences facilitate to distinguish one species from another [23]. Fluorescence microscopy places great emphasis on luminophores [24]. In past, the fluorescent dyes acridine orange and rhodamine C were widely used for staining the structure of parasites [25,26], but nowadays other synthetic dyes began to appear for the morphological study of biological organisms [27,28]. In recent years, benzanthrone luminophores have gained popularity and their use enables the detection of specific lipids and proteins [29,30]. Previous studies confirmed that benzanthrone luminophores are able to stain biological materials [30-32]. Every time when we think about luminophores, the photobleaching process has to be considered. Photobleaching is an irreversible process in which the fluorophore gradually fades [33]. Photofading occurs as a static process after many cycles of absorption and emission of photons. As a result of photofading, during the investigation of the sample, fading of the sample is observed and the fluorescence intensity drops, which means that the quality of the obtained result begins to gradually disappear [34,35]. Photofading could be considered as the main property of luminophores, as it affects the total number of emitted photons and the quality of the obtained result. As a result, more and more luminophores are continuously being synthesized in which attempts are made to slow down the photofading process [36]. Although staining with benzanthrone luminophores significantly reduces sample preparation time, a specific benzanthrone luminophore must be selected for each group of organisms [37].

Materials and methods

Information on the usage of the benzanthrone luminophores for rapid and high-resolution imaging of parasites by CLSM was gathered by searching in the International online databases Web of Science, ScienceDirect, PubMed (all fields), and Scopus (title, abstract, and keywords) were searched for all published data on the topic. The databases were searched for all published studies in English, from 2000 to 1 November 2023. The search results from the four databases were combined and duplicates were excluded. Eligible studies were selected based on the title and abstract.

Results

In a total, five relevant articles and one monography were identified during the literature search. First articles on utilisation of the benzanthrone luminophores for examination of the parasites are dated by 2018 year and the most recent publication is dated by 2021 year. The following parasite species were studied using the benzanthrone luminophores and confocal laser scanning microscopy: please refer Table 1 below listing articles, which include original research on benzanthrone luminophore utilisation for various parasite examination.

Table 1 – parasite species, luminophore and fixative used in the studies

Study	Parasite species	Luminophore	Fixation
Kirjusina et al., 2018 [7]	Fasciolidae	AZP5	96% ethanol
Kirilova et al., 2018 [21]	*Prosotocus confusus* ad.	P8, AM1, AM2, AM4, AM16	96% ethanol AFA
	Diplostomum spathaceum mtc.	P8, AM1, AM2, AM4, AM16	96% ethanol
	Diplodiscus subclaviatus ad.	P8, AM1, AM2, AM4, AM16	Carnoy's solution
Gavarane et al., 2018 [6]	*Trichinella britovi* larvae	P13	70% ethanol 96,6% ethanol, frozen storage in animal muscle 1-5 years
Gavarāne et al., 2019 [22]	*T. britovi* larvae	AZM	70% ethanol Bouin's solution Carnoy's solution AFA
	T. spiralis larvae	AZM	70% ethanol Bouin's solution Carnoy's solution AFA
Gavarane et al., 2020 [37]	*Diplostomum* sp.mtc.	AM2	96% ethanol
	Diplostomum sp. mtc.	AM2223	70% ethanol
	T. britovi larvae	AZP4	Bouin's solution
	Camallanus lacustris	AZP4	96% ethanol
	Dactylogyrus sp.	AZP4	96% ethanol
	Parafasciolopsis fasciolaemorpha ad.	AZP5	70% ethanol Carnoy's solution
	P. fasciolaemorpha ad.	AZR	70% ethanol
	P. fasciolaemorpha ad.	EAM1	70% ethanol
	P. confusus ad.	P10	AFA
Rubenina et al., 2021 [38]	*P. fasciolaemorpha* ad.	AZPP	70% ethanol 96% ethanol Bouin's solution Carnoy's solution AFA 10% Neutral buffered formalin
	P. fasciolaemorpha ad.	AM323	70% ethanol 96% ethanol Bouin's solution Carnoy's solution AFA 10% Neutral buffered formali

During the studies, mainly the parasite species of the Trematode class were investigated: species from a homothermic host as well as species of parasites from poikilothermic hosts [7, 20, 37-38]. All selected trematode parasite species are endoparasites. They are an important factor influencing the dynamics of wild populations [39]. Even more so, a high intensity of invasion and a wide variety of parasite species can significantly affect the health of the host [40].

Discussion

CLSM is widely used in the study of the morphological and physiological structure of various species, especially for fixed samples of trematodes. Moreover, the various studies and attempts to improve the properties of different luminophores provide opportunities to visualize the morphological features of the parasite's body surface, the organ systems of various trematode species in the adult and larval stages, especially the digestive and reproductive systems. Systematic studies of flatworm parasites focus on the detailed study of structures even on the surface of nutrient openings, reproductive system, excretory system and glands, possible functions of sensory organs and their arrangement on the surface, shape and types of arrangement of trematode spikes [41,42]. The results of the study [21] showed that using 488 nm (with a 500-655 nm filter) laser excitation, it was possible to achieve a 23x smaller autofluorescence signal, compared to 405 nm (with a 425-580 nm filter) wavelength excitation. When autofluorescence was evaluated, different Region of Interests (ROIs) were selected and the selected ROIs were compared to the background ROIs. Based on the obtained data, 488 nm laser with FITC filter (500-550 nm) and 638 nm laser with Cy5 filter (662-737 nm) were the most suitable lasers for suppressing unwanted autofluorescence. On the other hand, in the study with freshwater trematodes [38], a laser with a wavelength of 405 nm was not used, because it induces autofluorescence of the samples. In previous studies, localization of benzanthrone luminophores in model membranes was detected by Förster resonance energy transfer and red-edge absorption shift [29-30]. The hermaphrodite generation of trematodes is characterized by a body wall musculature consisting of three layers: annular, striated and diagonal [43,44]. Kirilova et al. [21] and Rubenina et al. [38] confirmed with their study results that all three characteristic layers of the musculature of the trematode body wall were observed as the developed protocol does not target any specific organ system or skeletal muscle layer. Although, it is possible that by modifying the staining protocol, it would be possible to observe other muscle fiber groups. Relatively complex staining methods are used for studies of muscle layers, for example, Krupenko [19] used a 4% paraformaldehyde solution in PBS as a fixative and stained *D. subclaviatus* with TRITC-conjugated phalloidin. Phalloidin binds to polymeric and oligomeric forms of actin [45]. The standard fluorescent actin staining protocol together with CLSM has been used several times to investigate the muscular system of trematodes [13,14; 46-49]. Preparation of the sample in this case requires one or even more than two days, but as a result, the smallest muscle fibers are highlighted, thus highlighting the most characteristic features of the species. With research-developed staining protocols [7, 21,22, 38], results can be obtained within the first two hours. When the first attempts were made to stain freshwater trematodes, the obtained results did not show the whole-body muscle structure, but the muscular pharynx, mouth and abdominal suckers were marked. On the other hand, in the study with *P. fasciolaemorpha,* a more detailed body muscle structure was observed, visualizing the annular, diagonal and striated musculature layers [7, 38].

The front part of the Trematode body is covered by spines with larger teeth, while in the lower part of the body, spines with smaller teeth or even spines with 2-3 peaks are found [50]. Kirilova et al. [21] and Rubenina et al. [38] in their studies observed that the front part of the parasite’s body is more densely covered with spines than the lower part of the body. Although Krupenko & Dobrovolskij [13] concluded that the shape of the spikes, the number of teeth, etc. not detectable only by the CLSM method, the results of their studies confirmed the opposite. The AZPP luminophore and the CLSM method provided information on the size of the spikes, the number of teeth and their shape [38].

The obtained results showed that due to its high lipophilicity, P8 can enter the hydrophobic regions of the membrane. The luminophore P8 is in the phospholipid head region, although other luminophore binding sites were closer to the membrane surface. Ryzhova and co-authors [30] confirmed that dyes AM2 and AM4 have lower

lipid binding abilities than P8 [21] due to their higher polarity.

The staining protocols developed in the study were not intended for staining and visualization of a specific organ system, therefore, it was checked whether any part of the nervous system would be observed [21, 38]. In the obtained results, when staining trematodes with the synthesized AZPP, AM323, AM1, AM2, AM4, AM16 and P8 benzanthrone luminophores, none of the parts of the nervous system were observed. During the study, the synthesized benzanthrone luminophores in the polar solvents showed fluorescence in the red spectrum region, however, stained samples had a fluorescence shift in the shorter wavelength region [21, 38]. This could be due to more hydrophobic conditions (higher number of lipids, dehydration with ethanol). Adjacent luminescence can be produced by a chemical fixative. For example, using a mixture of formalin-containing chemical fixatives, more intense cellular luminescence in the yellow-green region of the spectrum can be observed [51]. *T. spiralis* and *T. britovi* species of *Trichinella* genus were selected during the study conducted by Gavarane et al. [6] and Gavarane et al. [22]. Both *Trichinella* species are the causative agents of trichinosis, which is dangerous for humans and animals [52,53]. Due to morphological similarities, it has become very challenging to identify isolates of *Trichinella* species to the species level, therefore several biochemical and molecular methods have been developed over the years, thus facilitating the identification of species and genotypes [54]. Almost all cells and tissues are capable of fluorescing near ultraviolet radiation of the visible spectrum. To study the synthesized fluorescent luminophores, the fluorescence signal must be separated from the luminophore and the autofluorescence signal. The easiest way to do this is to choose the wavelength that corresponds to the absorption. Typically, the autofluorescence excitation region is around 400 nm and the 488 nm absorbance was selected in the study [6], suppressing the autofluorescence signal relative to the tracer fluorescence signal [55,56]. Gavarane et al. [6] found that the most suitable chemical fixative for *Trichinella* larvae obtained from animal musculature and fixed, which are then examined by CLSM, is Bouin's fixative. As a result, detailed data on the morphology of the larva and its arrangement were obtained. *Trichinella* larvae studies always analyse larval morphology. One of the features by which the sex of trichinella larvae can be distinguished is the length of the rectum. In male larvae, the average length of the rectum is 40 μm to 50 μm, but in female larvae it is almost half that at 17 μm to 35 μm. During the study, Gavarane et al. [22] developed the staining protocol, which is suitable for determining the sex of the Trichinella larva by measuring the length of the rectum. The results of the study showed that the length of the rectum in male T. britovi larvae is 41.08 ± 4.26 μm SD and in T. spiralis larvae – 46.08 ± 2.95 μm SD; for female larvae 21.19 ± 2.45 μm SD and 20.55 ± 1.48 μm SD. The obtained research data agree with the data of other studies and confirm that the length of the rectum of males is twice that of females [57-59]. By developing new methods for determining the sex of the parasite, the obtained data would be useful in controlling the reproductive strategy of the parasite population [60].

The larval cuticle consists of three or more outer layers that are made of collagen and other components. The epidermis or hypodermis forms the outer cuticle layer and this layer is acellular [61]. The obtained results showed a high fluorescence signal in the cuticle of larvae [6, 22]. The cause of the high fluorescence signal in the cuticle may be the accumulation of lipids in the epicuticle of Trichinella larvae, as lipids are the energy source for the survival of the parasite in the host's muscle cells [62]. In general, the body of Trichinella is covered by a ridged cuticle [63] and during the study [22] it was observed that there are differences between the cuticle of *T. spiralis* and *T. britovi* larvae. In T. spiralis larvae, striation, so-called "pseudo-segmentation", was observed, while no transverse lines or striations were observed in the cuticle of *T. britovi* [22]. When staining the samples with AZM, an esophagus consisting of a single-layered epithelium with a basement membrane on the basal side was also observed [6, 22]. Other studies have confirmed that four types of epithelia are observed in the esophagus of *Trichinella* and that some of the epithelial cells are myoepithelium, which provides esophageal peristalsis [64]. Different epithelial cells were not observed in Gavarane et al. [6, 22] studies, however, there is a possibility that they could be observed using CLSM if the staining protocol is optimized. In total 6 various chemical fixatives have been used for sample preparation. Articles confirmed, chemical fixation

step of the samples has an impact on imaging results as well. At least fifteen new benzanthrone luminophores had been synthesized and several staining protocols had been developed for the study of Nematoda phylum and Trematoda class species. The articles confirmed photobleaching factor, therefore, use of laser wavelengths in descending order is recommended. Interestingly that trematode's thickness has an important role in staining protocol development as thicker samples require dehydration step by 100% xylene.

All articles demonstrated that benzanthrone luminophores are useful for rapid and high-resolution imaging of the parasites. Basically, a new staining protocol should be developed every time when a new benzanthrone is synthetised as high-resolution imaging results are mandatory criteria for rapid object examination. However, results of all studies demonstrated that all benzanthrone luminophores are not equal for all groups of parasites.

Conclusions

Considering the results of literature, benzanthrone luminophores with CLSM can be used to: efficiently and rapid exam various Nematoda and Trematoda species; to study frozen larvae in animal musculature, to study samples stored in 96,6% ethanol or to study larvae that have been isolated from recently collected animal musculature within a day and fixed; to differentiate larvae of the *T. spiralis* and *T. britovi* species, to determine the sex of *T. spiralis* and *T. britovi* larvae based on the length of the larva's rectum length. Developed staining protocols allow to study the anatomical and muscular arrangement within the parasite's body.

Gratitude

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References

1 Zhang, Y., Design and Application of Receptor-Targeted Fluorescent Probes Based on Small Molecular Fluorescent Dyes [Text] / Zhang, Y., Li, S., Xu, H. // Bioconjugate Chem. - 2021. - No.32(1). -P. 4–24.

2 Makin, O.S., Structures for amyloid fibrils [Text] / Makin, O.S. & Serpell, L.C. // FEBS J. -2005. - No.272. -P.5950-5961.

3Yang, X., bindingstudies of a solvatochromic fluorescence probe 3-methoxybenzanthrone [Text] / Yang, X., Liu, W.H., Jin, W.J., Shen, G.L. & Yu, R.Q. // Spectrochim Acta A. -1999. - No.55. -P.2719–2727.

4 Zhytniakivska, O., Newly synthesized benzanthrone derivatives as prospective fluorescent membrane probes [Text] / Zhytniakivska, O., Trusova, V., Gorbenko, G., Kirilova, E., Kalnina, I., Kirilov, G. & Kinnunen, P. // J. Lumin. -2014. - No.146. -P.307– 313.

5 Kirilova, E.M., Spectroscopic Study of Benzanthrone 3-N-Derivatives as New Hydrophobic Fluorescent Probes for Biomolecules [Text] / Kirilova, E.M., Kalnina, I., Kirilov, G.K. & Meirovics, I. // J. Fluoresc. -2008. - No.18. -P.645– 648.

6 Gavarane, I., Staining of economically important parasitic nematodes by developed derivatives of benzanthrone luminophore [Text] / Gavarane, I., Mezaraupe, L., Rubenina, I., Kirjusina, M. & Kirilova, J. // 18th International Multidisciplinary Scientific GeoConference SGEM 2018, section Advances in Biotechnology. – 2018. -P. 581–587.

7 Kirjusina, M., Application of novel synthesized luminophore AZP5 for efficient staining of Trematoda: Fasciolidae parasites [Text] / Kirjusina, M., Gavarane, I., Mezaraupe, L., Kecko, S. & Kirilova, E. Int. Multidiscip. Sci. GeoConference SGEM. -2018. - No.18. -P.27–34.

8 Kirilova, E., Novel dye for detection of callus embryo by confocal laser scanning fluorescence microscopy [Text] / Kirilova, E., Mickevica, I., Mezaraupe, L., Puckins, A., Rubenina, I., Osipovs, S., Kokina, I., Bulanovs, A., Kirjusina, M. & Gavarane, I. // Luminescence. -2019. - No.34. -P.353–359.

9 Van der Berg, A., Micro total analysis systems [Text] / Van der Berg, A., Olthuis, W. & Bergveld, P. // Dordrecht, Springer. -2020.

10 Roda, A., Molecular luminescence imaging [Text] / Roda, A., Guardigli, M., Ziessel, R., Mirasoli, M., Michelini, E. & Musiani, M. // Microchemical Journal, - 2007. - No.585(1). -P.5-12.

11 Mochalova, N.V., Dicrocoelium lanceatum (Trematoda, Dicrocoelidae): the study of the neuromuscular system [Text] / Mochalova, N.V., Terenina, N.B., Kreshchenko, N.D., Yashin, V.A., Nefedova, D.A., Nikogoyan, M.A., Petrosvan, A.R. & Moysesyan, S.O. // Theory and practice of parasitic disease control. International Scientific Conference, Moscow. -2019.

12 Terenina, N.B., The New Data on the Serotonin and FMRFamide Localization in the Nervous System of Opisthorchis felineus Metacercaria [Text] / Terenina, N.B., Kreshchenko, N.D., Mochalova, N.V., Nefedova, D., Voropaeva, E.L., Movsesyan, S.O., Demiaszkiewicz, A., Yashin, V.A. & Kuchin A.V. // Acta Parasitol. -2020. - No.5(2). -P.61-374.

13 Krupenko, D., Morphological framework for attachment and locomotion in several Digenea of the families Microphallidae and Heterophyidae [Text] / Krupenko, D. & Dobrovolskij, A.A. // Parasitol. Res. -2018. - No.117.-P.3799–3807.

14 Krupenko, D.Y., Oral sucker in Digenea: Structure and muscular arrangement [Text] / Krupenko, D.Y. // Zoomorphology. -2019. - No.138. -P.29–37.

15 Selbach, C., Hidden parasite diversity in a European freshwater system [Text] / Selbach, C., Soldánová, M., Feld, C.K., Kostadinova, A. & Sures, B. // Sci Rep. -2020. - No.10. -P.2694.

16 Morand, S., (macro-) Evolutionary ecology of parasite diversity: From determinants of parasite species richness to host diversification [Text] / Morand, S. // Int. J. Parasitol. Parasites Wildl. - 2015. - No.4(1). - P.80-87.

17 Betts, A., High Parasite Diversity Accelerates Host Adaptation and Diversification. [Text] / Betts, A., Gray, C., Zelek, M., MacLean, R.C. & King, K.C. // Science. - 2019. - No.360(6391). - P.907–911.

18 Rehorek, S.J., How to Use Taxonomic Principles in a Non-Scientific Setting to Teach Hierarchical Thinking [Text] / Rehorek, S.J., Shotwell, M.A. The American Biology Teacher. -2018. - No.80(6). -P.446–450.

19 Krupenko, D.Y., Muscle system of Diplodiscus subclaviatus (Trematoda: Paramphistomida) cercariae, pre-ovigerous, and ovigerous adults [Text] / Krupenko, D.Y. // Parasitol. Res. -2014. - No.113. -P.941–952.

20 Marques, J.S., New insights on the morphology of a digenean parasite (Digenea: Brachylaimidae, Brachylaima mazzantii (Travassos, 1927)) using confocal laser scanning microscopy [Text] / Marques, J.S., Rocha, B.M., Manso, P.P.A. & D'Ávila, S. // Zoosystema. -2017. - No.39(4). -P.449-462.

21 Kirilova, E., Novel luminescent dyes for confocal laser scanning microscopy used in Trematoda parasite diagnostics [Text] / Kirilova, E., Kecko, S., Mežaraupe, L., Gavarāne, I., Pučkins, A., Mickeviča, I., Rubeniņa, I., Osipovs, S., Bulanovs, A., Pupiņš, M. & Kirjušina, M. // Acta Biochimica Polonica. -2018 - No.65(3). -P.449-454.

22 Gavarāne, I., A Simple and Rapid Staining Technique for Sex Determination of Trichinella Larvae Parasites by Confocal Laser Scanning Microscopy [Text] / Gavārane, I., Kirilova, E., Rubeniņa, I., Mežaraupe, L., Osipovs, S., Deksne, G., Pučkins, A., Kokina, I., Bulanovs, A. & Kirjušina M. // Microscopy and Microanalysis. -2019. -P.1-7.

23 Qazi, F., Real-time detection and identification of nematode eggs genus and species through optical imaging [Text] / Qazi, F., Khalid, A., Poddar, A., Tetienne, J.P., Nadarajah, A., Aburto-Medina, A., Shahsavari, E., Shukla, R., Prawer, S., Ball, A.S. & Tomljenovic-Hanic, S. // Sci Rep. -2020. - No.10. -P.7219.

24 Ndao, M., Diagnosis of parasitic diseases: old and new approaches [Text] / Ndao, M. // Interdiscip. Perspect. Infect. Dis. - 2009. - P.278246.

25 Geller, E.R., Study of the morphogenesis of Trichinella spiralis in a fluorescent microscope [Text] / Geller, E.R. & Timonov, E.V. // Wiadomosci Parazytologiczne. -1969. - No.15(5/6). - P.522-525.

26 Stankiewicz, M., Supravital staining of eosinophils [Text] / Stankiewicz, M., Jonas, W., Hadas, E., Cabaj, W. & Douch, P.G.C. // Int. J. Parasitol. -1996. - No.26(4). - P.445-446.

27 Dapson, R.W., The history, chemistry and modes of action of carmine and related dyes [Text] / Dapson, R.W. // Biotech. Histochem. -2007. - No.82. -P.173–187.

28 Fakhar, M., Phenazopyridine as an innovative stain for permanent staining of trematodes [Text] / Fakhar, M. & Ghobaditara, M. // Trop. Parasitol. -2016. - No.6:86–88.

28 Zhytniakivska, O., Location of novel benzanthrone dyes in model membranes as revealed by resonance energy transfer [Text] / Zhytniakivska, O., Trusova, V., Gorbenko, G., Kirilova, E., Kalnina, I., Kirilov, G., Molotkovsky, J., Tulkki, J. & Kinnunen, P. J. // Fluoresc. -2014. - No.24.-P.899–907.

29 Ryzhova O., Novel benzanthrone probes for membrane and protein studies [Text] / Rizhao, O., Vus, K., Trusova, V., Kirilova, E., Kirilov, G., Gorbenko, G. & Kinnunen, P. // Methods Appl Fluores. -2016. - No.4. -P.034007.

30 Kalnina, I., Fluorescent probe ABM for screening gastrointestinal patient's immune state [Text] / Kalnina, I., Klimkane, L., Kirilova, E., Toma, M.M., Kizane, G. & Meirovics, I. // J. Fluoresc. -2007. - No.17. -P.619–625.

31 Trusova, V.M., Novel Benzanthrone Aminoderivatives for Membrane Studies [Text] / Trusova, V.M., Kirilova, E., Kalnina, I., Kirilov, G, Zhytniakivska, O., A., Fedorov, P.V. & Gorbenko, G. P., J Fluoresc. -2012. - No.22. -P.953–959.

32 Eggeling, C., C. A. M. Seidel in Applied fluorescence in chemistry, biology and medicine [Text] / (Eds.: W. Rettig, B. Strehmel, M. Schrader, H. Seifert), Eggeling, C., Widengren, J. & Rigler, R. Springer, Berlin, -1999. -P.193.

33 Zondervan, R., Photobleaching of Rhodamine 6G in Poly (vinyl alcohol) at the Ensemble and Single-Molecule Levels [Text] / Zondervan, R., Kulzer, F, Kolchenk, M.A. & Orrit, M. J. // Phys. Chem. A. -2018. -P.1657-1665.

34 Han, D., Electrochemiluminescence Loss in Photobleaching [Text] / Han, D., Goudeau, B., Manojlovic, D., Jiang, D., Fang, D. & Sojic, N. // Angew. Chem. Int. Ed. -2021. - No.60. -P.7686–7690.

35 Demchenko, A.P., Photobleaching of organic fluorophores: quantitative characterization, mechanisms, protection [Text] / Demchenko, A.P. // Methods Appl. Fluoresc. -2020. - No.8. -P.022001.

36 Gavarane, I., Simple and rapid luminiscent staining protocols in Helmintology [Text] / Gavarane, I., Kirilova, E., Rubenina, I., Osipovs, S., Mezaraupe, L., Puckins, A. & Kirjusina M. // [Monograph] Published by Daugavpils University "Saule", Daugavpils. - 2020. - P.1-131.

37 Rubenina, I., Comparison of the Benzanthrone Luminophores: They Are Not Equal for Rapid Examination of Parafasciolopsis fasciolaemorpha (Trematoda: Digenea) [Text] / Rubenina, I., Gavarane, I., Kirilova, E., Mezaraupe, L. & Kirjusina, M. // Biomolecules. - 2021. - No.11(598). -P.1-15. https://doi.org/10.3390/biom11040598

38 Kołodziej-Sobocinska, M., Increased parasitic load in captive-released European bison (Bison bonasus) has important implications for reintroduction programs [Text] / Kołodziej-Sobocinska, M., Demiaszkiewicz, A.W., Pyziel, A.M. & Kowalczyk, R. // EcoHealth. -2018. - No.15(2). -P.467–471.

39 Filip-Hutsch, K., Gastrointestinal Helminths of a European Moose Population in Poland [Text] / Filip-Hutsch, K., Czopowicz, M., Barc, A. & Demiaszkiewicz, A.W. // Pathogens. -2021. - No.10. -P.456.

40 Jurberg, A.D., Trematode embryology: a new method for whole-egg analysis by confocal microscopy [Text] / Jurberg, A.D., Pascarelli, B.M., Pelajo-Machado, M., Maldonado, A. Jr., Mota, E.M. & Lenzi, H.L. // Dev Genes Evol. -2008. - No.218. -P.267–271.

41 Borges, N., Molecular characterization and confocal laser scanning microscopic study of Pygidiopsis macrostomum (Trematoda: Heterophyidae) parasites of guppies Poecilia vivipara [Text] / Borges, N., Costa, V.S., Mantovani, C., Barros, E., Santos, E.G.N., Marfa, C.L. & Santos, C.P. // J Fish Dis. -2017. - No.40.-P.191–203.

42 Ginetsinskaya, T., Trematodes, Their Life Cycles, Biology and Evolution [Text] / New Delhi: Amerind Publ. Co. Pvt. Ltd. -1988.

43 Galaktionov, K.V., Biology and Evolution of Trematodes. An Essay on the Biology, Morphology, Life Cycles, Transmission, and Evolution of Digenetic Trematodes [Text] / Galaktionov, K.V. & Dobrovolskij, A.A. // London: Kluwer Academic Publishers. -2003. -P.17.

44 Oda, T., Position and orientation of phalloidin in F-actin determined by x-ray fiber diffraction analysis [Text] / Oda, T., Namba, K. & Maéda, Y. // Biophys J. -2005. - No.88. -P.2727–2736.

45 Mair, G.R., Organization of the musculature of schistosome cercariae [Text] / Mair, G.R., Maule, A.G., Fried, B., Day, T.A. & Halton, D.W. // J Parasitol. -2003. - No.89(3). -P.623–625.

46 Shebelova, I.Y., Inducing conjugate chlorination of alkenes with sulfur dichloride [Text] / Shebelova, I.Y., Sazhin, A.A., Bodrikov, I.V. & Barkhash, V.A. // Russian J. Org. Chem. -2000. - No.36. -P. 597.

47 Krupenko, D.Y., Somatic musculature in trematode hermaphroditic generation [Text] / Krupenko, D.Y. & Dobrovolskij, A.A. // BMC Evol. Biol. -2015. - No.15. -P.189.

48 Petrov, A., Muscle architecture during the course of development of Diplostomum pseudospathaceum Niewiadomska, 1984 (Trematoda, Diplostomidae) from cercariae to metacercariae [Text] / Petrov, A. & Podvyaznaya, I. // J Helminthol. -2016. - No.90(3). -P.321–336.

49 Køie, M., Stereoscan studies of cercariae, metacercariae, and adults of Cryptocotyle lingua (Creplin 1825) Fischoeder 1903 (Trematoda: Heterophyidae) [Text] / Køie, M. // J Parasitol. -1977. - No.63(5). -P.835–839.

50 Alfano, R.R., Laser induced fluorescence spectroscopy from native cancerous and normal tissue [Text] / Alfano, R.R., Tata, D.B., Corsero, J., Tomashefsky, P., Longo, F.W. & Alfano, M.A. // IEEE J Quantum Elect. -1984. - No.20. -P.1507–1511.

51 Rozycki, M., Analysis of a Trichinellosis Outbreak in Poland after Consumption of Sausage Made of Wild Boar Meat [Text] / Rozycki, M., Korpysa-Dzirba, W., Belick, A., Pelec, T., Mazurek, J. & Cencek, T. // Meat. J. Clin. Med. -2022. - No.11. -P.485.

52 Tso, M., Trichinellosis & Heart. In Neglected Tropical Diseases and other Infectious Diseases affecting the Heart [Text] / Tso, M. // Academic Press. -2022. -P.117-124.

53 Zarlenga, D., Trichinella species and genotypes [Text] / Zarlenga, D., Thompson, P. & Pozio, E. // Vet. Sci. Res. J. -2020. - No.133. -P.289–296.

54 Schnell, S.A., Reduction of lipofuscinlike autofluorescence in fluorescently labeled tissue [Text] / Schnell, S.A., Staines, W.A. & Wessendorf, M.W. // J Histochem Cytochem. -1999. - No.47. -P.719–730.

55 Neumann, M., Simple method for reduction of autofluorescence in fluorescence microscopy [Text] / Neumann, M. & Gabel, D. // J Histochem Cytochem. -2002. - No.50. -P.437–439.

56 Kozek, W.J., Trichinella spiralis: Morphological characteristics of male and female intestine-infecting larvae [Text] / Kozek, W.J. // Exp Parasitol. -1975. - No.37. -P.380–387.

57 Liu, Z.M., Differentiation of the sex of Trichinella larvae collected in Changchun [Text] / Liu, Z.M., Wang, C. & An, C.L. // Chinese Journal of Parasitology & Parasitic Diseases. -1991. - No.9(3). -P.223–225.

58 Pozio, E., Trichinella. Liu D. (Ed.), Molecular detection of foodborne pathogens [Text] / Pozio, E. & La Rosa, G. // CRC Press Taylor and Francis Group, Boca Raton, London, New York. -2010. -P.851–863.

59 Pires-da Silva, A., Evolution of the control of sexual identity in nematodes [Text] / Pires-da Silva, A. // Semin Cell Dev Biol. -2007. -№.18. -P.362–370.

60 Lichtenfels, J.R., Comparison of three subspecies of Trichinella spiralis by scanning electron microscopy [Text] / Lichtenfels, J.R., Murrell, K.D. & Pilitt, P.A. // J. Parasitol. -1983. - No.69. -P.1131–1140.

61 Gounaris, K., Structural organisation and lipid composition of the epicuticular accessory layer of infective larvae of Trichinella spiralis [Text] / Gounaris, K., Smith, V.P. & Selkirk, M.E. // Biochim Biophys Acta. -1996. - No.1281. -P.91–100.

62 Hetherington, D.C., Comparative studies on certain features of nematodes and their significance [Text] / Hetherington, D.C. // Illinois biological monographs. -1924. - No.8(2). -P.1-62.

63 Takahashi, Y., Biology of Trichinella [Text] / In Trichinella and Trichinellosis, Academic Press. -2021. -P.77-101.

References

1 Zhang, Y., Li, S., Xu, H. (2021). Design and Application of Receptor-Targeted Fluorescent Probes Based on Small Molecular Fluorescent Dyes. Bioconjugate Chem. 32(1), 4–24. https://doi.org/10.1021/acs.bioconjchem.0c00606

2 Makin, O.S. & Serpell, L.C. (2005). Structures for amyloid fibrils FEBS J. 272, 5950-5961. 10.1111/j.1742-4658.2005. 05025.x

3 Yang, X., Liu, W.H., Jin, W.J., Shen, G.L. & Yu, R.Q. (1999). bindingstudies of a solvatochromic fluorescence probe 3-methoxybenzanthrone. Spectrochim Acta A. 55, 2719–2727. https://doi.org/10.1016/S1386-1425(99)00161-4

4 Zhytniakivska, O., Trusova, V., Gorbenko, G., Kirilova, E., Kalnina, I., Kirilov, G. & Kinnunen, P. (2014). Newly synthesized benzanthrone derivatives as prospective fluorescent membrane probes. J. Lumin. 146,307-313. DOI: 10.1016/j.jlumin.2013.10.015

5 Kirilova, E.M., Kalnina, I., Kirilov, G.K. & Meirovics, I. (2008). Spectroscopic Study of Benzanthrone 3-N-Derivatives as New Hydrophobic Fluorescent Probes for Biomolecules. J. Fluoresc. 18,645–648. DOI: 10.1007/s10895-008-0340-3

6 Gavarane, I., Mezaraupe, L., Rubenina, I., Kirjusina, M. & Kirilova, J. (2018). Staining of economically important parasitic nematodes by developed derivatives of benzanthrone luminophore. 18th International Multidisciplinary Scientific GeoConference SGEM 2018, section Advances in Biotechnology. 581–587. https://doi.org/10.5593/sgem 2018/6.2

7 Kirjusina, M., Gavarane, I., Mezaraupe, L., Kecko, S. & Kirilova, E. (2018). Application of novel synthesized luminophore AZP5 for efficient staining of Trematoda: Fasciolidae parasites. Int. Multidiscip. Sci. GeoConference SGEM. 18, 27–34. https://doi.org/10.5593/sgem 2018/6.2

8 Kirilova, E., Mickevica, I., Mezaraupe, L., Puckins, A., Rubenina, I., Osipovs, S., Kokina, I., Bulanovs, A., Kirjusina, M. & Gavarane, I. (2019). Novel dye for detection of callus embryo by confocal laser scanning fluorescence microscopy. Luminescence. 34, 353–359.

9 Van der Berg, A., Olthuis, W. & Bergveld, P. (2020). Micro total analysis systems. Dordrecht, Springer. https://doi.org/10.1007/978-94-017-2264-3

10 Roda, A., Guardigli, M., Ziessel, R., Mirasoli, M., Michelini, E. & Musiani, M. (2007). Molecular luminescence imaging. Microchemical Journal. 585(1),5-12. https://doi.org/10.1016/j.microc.2006.04.010

11 Mochalova, N.V., Terenina, N.B., Kreshchenko, N.D., Yashin, V.A., Nefedova, D.A., Nikogoyan, M.A., Petrosvan, A.R. & Moysesyan, S.O. (2019). Dicrocoelium lanceatum (Trematoda, Dicrocoelidae): the study of the neuro-muscular system. Theory and practice of parasitic disease control. International Scientific Conference, Moscow.

12 Terenina, N.B., Kreshchenko, N.D., Mochalova, N.V., Nefedova, D., Voropaeva, E.L., Movsesyan, S.O., Demiaszkiewicz, A., Yashin, V.A. & Kuchin A.V. (2020). The New Data on the Serotonin and FMRFamide Localization in the Nervous System of Opisthorchis felineus Metacercaria. Acta Parasitol. 5(2),61-374.

13 Krupenko, D. & Dobrovolskij, A.A. (2018). Morphological framework for attachment and locomotion in several Digenea of the families Microphallidae and Heterophyidae. Parasitol. Res. 117, 3799–3807.

14 Krupenko, D.Y., (2019). Oral sucker in Digenea: Structure and muscular arrangement. Zoomorphology. 138, 29–37.

15 Selbach, C., Soldánová, M., Feld, C.K., Kostadinova, A. & Sures, B. (2020). Hidden parasite diversity in a European freshwater system. Sci Rep. 10, 2694. https://doi.org/10.1038/s41598-020-59548-5

16 Morand, S., (macro-) (2015). Evolutionary ecology of parasite diversity: From determinants of parasite species richness to host diversification. Int. J. Parasitol. Parasites Wildl. 4(1), 80-87. https://doi.org/10.1016/j.ijppaw.2015.01.001.

17 Betts, A., Gray, C., Zelek, M., MacLean, R.C. & King, K.C. (2019). High Parasite Diversity Accelerates Host Adaptation and Diversification. Science. 360(6391),907–911. doi: 10.1126/science.aam9974

18 Rehorek, S.J., Shotwell, M.A. (2018). How to Use Taxonomic Principles in a Non-Scientific Setting to Teach Hierarchical Thinking. The American Biology Teacher. -80(6),446–450.

19 Krupenko, D.Y., (2014). Muscle system of Diplodiscus subclaviatus (Trematoda: Paramphistomida) cercariae, pre-ovigerous, and ovigerous adults. Parasitol. Res. -113, 941–952.

20 Marques, J.S., Rocha, B.M., Manso, P.P.A. & D'Ávila, S. (2017). New insights on the morphology of a digenean parasite (Digenea: Brachylaimidae, Brachylaima mazzantii (Travassos, 1927)) using confocal laser scanning microscopy. Zoosystema. 39(4),449-462. https://doi.org/10.5252/z2017n4a1

21 Kirilova, E., Kecko, S., Mežaraupe, L., Gavarāne, I., Pučkins, A., Mickeviča, I., Rubeniņa, I., Osipovs, S., Bulanovs, A., Pupiņš, M. & Kirjušina, M. (2018). Novel luminescent dyes for confocal laser scanning microscopy used in Trematoda parasite diagnostics. Acta Biochimica Polonica. -65(3), 449-454. https://onlinelibrary.wiley.com/doi/abs/10.1002/bio.3616

22 Gavārane, I., Kirilova, E., Rubeniņa, I., Mežaraupe, L., Osipovs, S., Deksne, G., Pučkins, A., Kokina, I., Bulanovs, A. & Kirjušina M. (2019). A Simple and Rapid Staining Technique for Sex Determination of Trichinella Larvae Parasites by Confocal Laser Scanning Microscopy. Microscopy and Microanalysis. 1-7. doi:10.1017/S1431927619015046

23 Qazi, F., Khalid, A., Poddar, A., Tetienne, J.P., Nadarajah, A., Aburto-Medina, A., Shahsavari, E., Shukla, R., Prawer, S., Ball, A.S. & Tomljenovic-Hanic, S. (2020). Real-time detection and identification of nematode eggs genus and species through optical imaging. Sci Rep. 10, 7219.

24 Ndao, M., (1969). Diagnosis of parasitic diseases: old and new approaches. Interdiscip. Perspect. Infect. Dis. 278246.

25 Geller, E.R. & Timonov, E.V. (1969). Study of the morphogenesis of Trichinella spiralis in a fluorescent microscope. Wiadomosci Parazytologiczne.15(5/6), 522-525.

26 Stankiewicz, M., Jonas, W., Hadas, E., Cabaj, W. & Douch, P.G.C. (1996). Supravital staining of eosinophils. Int. J. Parasitol. 26(4), 445-446.

27 Dapson, R.W., (2007). The history, chemistry and modes of action of carmine and related dyes. Biotech. Histochem. 82,173–187.

28 Fakhar, M. & Ghobaditara, M. (2016). Phenazopyridine as an innovative stain for permanent staining of trematodes. Trop. Parasitol. 6:86–88.

28 Zhytniakivska, O., Trusova, V., Gorbenko, G., Kirilova, E., Kalnina, I., Kirilov, G., Molotkovsky, J., Tulkki, J. & Kinnunen, P. J. (2014). Location of novel benzanthrone dyes in model membranes as revealed by resonance energy transfer. Fluoresc. 24, 899–907.

29 Ryzhova, O., Vus, K., Trusova, V., Kirilova, E., Kirilov, G., Gorbenko, G. & Kinnunen, P. (2016). Novel benzanthrone probes for membrane and protein studies. Methods Appl Fluores. 4, 034007.

30 Kalnina, I., Klimkane, L., Kirilova, E., Toma, M.M., Kizane, G. & Meirovics, I. (2007). Fluorescent probe ABM for screening gastrointestinal patient's immune state. J. Fluoresc. 17,619–625.

31 Trusova, V.M., Kirilova, E., Kalnina, I., Kirilov, G, Zhytniakivska, O., A., Fedorov, P.V. & Gorbenko, G. P., (2012). Novel Benzanthrone Aminoderivatives for Membrane Studies. J Fluoresc. 22, 953–959.

32 Eggeling, C., Widengren, J. & Rigler, R. (1999). Seidel in Applied fluorescence in chemistry, biology and medicine. (Eds.: W. Rettig, B. Strehmel, M. Schrader, H. Seifert. Springer, Berlin, 193.

33 Zondervan, R., Kulzer, F, Kolchenk, M.A. & Orrit, M. J. (2018). Photobleaching of Rhodamine 6G in Poly (vinyl alcohol) at the Ensemble and Single-Molecule Levels. Phys. Chem. A. 1657-1665.

34 Han, D., Goudeau, B., Manojlovic, D., Jiang, D., Fang, D. & Sojic, N. (2021). Electrochemiluminescence Loss in Photobleaching. Angew. Chem. Int. Ed. 60, 7686–7690.

35 Demchenko, A.P., (2020). Photobleaching of organic fluorophores: quantitative characterization, mechanisms, protection. Methods Appl. Fluoresc. 8, 022001.

36 Gavarane, I., Kirilova, E., Rubenina, I., Osipovs, S., Mezaraupe, L., Puckins, A. & Kirjusina M. (2020). Simple and rapid luminiscent staining protocols in Helmintology. [Monograph] Published by Daugavpils University "Saule", Daugavpils. 1-131.

37 Rubenina, I., Gavarane, I., Kirilova, E., Mezaraupe, L. & Kirjusina, M. (2021). Comparison of the Benzanthrone Luminophores: They Are Not Equal for Rapid Examination of Parafasciolopsis fasciolaemorpha (Trematoda: Digenea). Biomolecules. 11(598),1-15. https://doi.org/10.3390/biom11040598

38 Kołodziej-Sobocinska, M., Demiaszkiewicz, A.W., Pyziel, A.M. & Kowalczyk, R. (2018). Increased parasitic load in captive-released European bison (Bison bonasus) has important implications for reintroduction programs. EcoHealth. 15(2),467–471.

39 Filip-Hutsch, K., Czopowicz, M., Barc, A. & Demiaszkiewicz, A.W. (2021). Gastrointestinal Helminths of a European Moose Population in Poland. Pathogens. -10, 456.

40 Jurberg, A.D., Pascarelli, B.M., Pelajo-Machado, M., Maldonado, A. Jr., Mota, E.M. & Lenzi, H.L. (2008). Trematode embryology: a new method for whole-egg analysis by confocal microscopy. Dev Genes Evol. 218, 267–271.

41 Borges, N., Costa, V.S., Mantovani, C., Barros, E., Santos, E.G.N., Marfa, C.L. & Santos, C.P. (2017). Molecular characterization and confocal laser scanning microscopic study of Pygidiopsis macrostomum (Trematoda: Heterophyidae) parasites of guppies Poecilia vivipara. J Fish Dis. 40, 191–203.

42 Ginetsinskaya, T., (1988). Trematodes, Their Life Cycles, Biology and Evolution. New Delhi: Amerind Publ. Co. Pvt. Ltd.

43 Galaktionov, K.V. & Dobrovolskij, A.A. (2003). Biology and Evolution of Trematodes. An Essay on the Biology, Morphology, Life Cycles, Transmission, and Evolution of Digenetic Trematodes. London: Kluwer Academic Publishers. 17.

44 Oda, T., Namba, K. & Maéda, Y. (2005). Position and orientation of phalloidin in F-actin determined by x-ray fiber diffraction analysis. Biophys J. 88,2727–2736.

45 Mair, G.R., Maule, A.G., Fried, B., Day, T.A. & Halton, D.W. (2003). Organization of the musculature of schistosome cercariae. J Parasitol. 89(3),623–625.

46 Shebelova, I.Y., Sazhin, A.A., Bodrikov, I.V. & Barkhash, V.A. (2000). Inducing conjugate chlorination of alkenes with sulfur dichloride. Russian J. Org. Chem. 36,597.

47 Krupenko, D.Y. & Dobrovolskij, A.A. (2015). Somatic musculature in trematode hermaphroditic generation. BMC Evol. Biol. 15,189.

48 Petrov, A. & Podvyaznaya, I. (2016). Muscle architecture during the course of development of Diplostomum pseudospathaceum Niewiadomska, 1984 (Trematoda, Diplostomidae) from cercariae to metacercariae. J Helminthol. 90(3),321–336.

49 Køie, M., (1977). Stereoscan studies of cercariae, metacercariae, and adults of Cryptocotyle lingua (Creplin 1825) Fischoeder 1903 (Trematoda: Heterophyidae). J Parasitol. 63(5),835–839.

50 Alfano, R.R., Tata, D.B., Corsero, J., Tomashefsky, P., Longo, F.W. & Alfano, M.A. (1984). Laser induced fluorescence spectroscopy from native cancerous and normal tissue IEEE J Quantum Elect. 20,1507–1511.

51 Rozycki, M., Korpysa-Dzirba, W., Belick, A., Pelec, T., Mazurek, J. & Cencek, T. (2022). Analysis of a Trichinellosis Outbreak in Poland after Consumption of Sausage Made of Wild Boar Meat. Meat. J. Clin. Med. 11,485.

52 Tso, M., (2022). Trichinellosis & Heart. In Neglected Tropical Diseases and other Infectious Diseases affecting the Heart. Academic Press. 117-124.

53 Zarlenga, D., Thompson, P. & Pozio, E. (2020). Trichinella species and genotypes. Vet. Sci. Res. J. 133,289–296.

54 Schnell, S.A., Staines, W.A. & Wessendorf, M.W. (1999). Reduction of lipofuscinlike autofluorescence in fluorescently labeled tissue. J Histochem Cytochem. 47,719–730.

55 Neumann, M. & Gabel, D. (2002). Simple method for reduction of autofluorescence in fluorescence microscopy. J Histochem Cytochem. 50,437–439.

56 Kozek, W.J., (1975). Trichinella spiralis: Morphological characteristics of male and female intestine-infecting larvae. Exp Parasitol. 37,380–387.

57 Liu, Z.M., Wang, C. & An, C.L. (1991). Differentiation of the sex of Trichinella larvae collected in Changchun. Chinese Journal of Parasitology & Parasitic Diseases. 9(3),223–225.

58 Pozio, E. & La Rosa, G. (2010). Trichinella. Liu D. (Ed.), Molecular detection of foodborne pathogens. CRC Press Taylor and Francis Group, Boca Raton, London, New York. 851–863.

59 Pires-da Silva, A., (2007). Evolution of the control of sexual identity in nematodes. Semin Cell Dev Biol. 18,362–370.

60 Lichtenfels, J.R., Murrell, K.D. & Pilitt, P.A. (1983). Comparison of three subspecies of Trichinella spiralis by scanning electron microscopy. J. Parasitol. 69,1131–1140.

61 Gounaris, K., Smith, V.P. & Selkirk, M.E. (1996). Structural organisation and lipid composition of the epicuticular accessory layer of infective larvae of Trichinella spiralis. Biochim Biophys Acta. 1281,91–100.

62 Hetherington, D.C., (1924). Comparative studies on certain features of nematodes and their significance. Illinois biological monographs. 8(2),1-62.

63 Takahashi, Y., (2021). Biology of Trichinella. In Trichinella and Trichinellosis, Academic Press. 77-101.