Commentary on the Habilitation Thesis

Strategies of Parasitism in Early Branching Apicomplexa

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Apicomplexa, consisting entirely of parasites occurring in a wide spectrum of invertebrates and vertebrates, cause human and animal diseases that represent a major world health problem with a considerable impact on the global economy. Apicomplexans represent one of the most successful groups of eukaryotic unicellular parasites, which have evolved unique adaptations for invading and surviving within hosts. Whilst the nature of the diseases caused by apicomplexans differs significantly, their common origin led them to share specific metabolic pathways unique to this group, and these may constitute potential targets for intervention. The knowledge of Apicomplexa, however, is mainly based on studies of etiologic agents of globally significant diseases (e.g. malaria, toxoplasmosis) belonging to evolutionary advanced (mostly intracellular) lineages. Nevertheless, the enormously diversified basal apicomplexan lineages restricted to invertebrate hosts, which have not received sufficient attention, are important from an evolutionary perspective due to their basal phylogenetic position. Recent studies have shown that apicomplexans evolved from photosynthetic ancestors. It is assumed that ancestral apicomplexans parasitised marine annelids and then spread to other marine invertebrate groups (crustaceans, turbellarians, echinoderms, etc.), followed by freshwater and terrestrial invertebrates and finally vertebrates. Our own and other published studies have shown that representatives of basal apicomplexan lineages exhibit an enormous diversity in cell dimension and architecture (correlating with their surrounding environment and parasitism strategy) and appear to be perfect examples of coevolution between parasites and their hosts. Therefore, with my co-workers and students, we focus on the poorly studied early branching Apicomplexa, which in various ways realise the intracellular, epicellular and extracellular parasitism in different organs and cavities of invertebrates and vertebrates. We have directed our research on blastogregarines, gregarines, agamococcidia, protococcidia and cryptosporidia that have

been hypothesised as early branching apicomplexans, with some of these (agamococcidia, protococcidia) recently revealed as sister lineages to medically important groups.

Presented habilitation thesis, summarising observations on representatives of diverse basal apicomplexan groups and placing individual findings in a broader context, offers a novel perspective on parasitism strategies in Apicomplexa. It presents a comprehensive work based on 16 publications, in which we used a combination of various methodological approaches, including standard parasitological and protistological procedures, *in vivo* and *in vitro* experimental assays, light and electron microscopy, histopathological methods, (immuno)cyto-/histochemistry, molecular biological and phylogenetic techniques. In summarising the publications produced for this thesis, as well as those closely related to the topic, I have tried to highlight the diversity of apicomplexan survival and parasitism strategies occurring in different environments. Two main aspects are discussed in detail, i.e. host-parasite interactions and parasite motility, as these represent a potential target for chemotherapeutic intervention in Apicomplexa. The study focused on identifying the structures and mechanisms responsible for the various modes of attachment to host cell/tissue, nutrient acquisition and motility in basal lineages and how these are modified in comparison to other Apicomplexa. The results presented herein support the hypothesis of Apicomplexa evolution progressing from myzocytotic predation (myzocytosis also called "cellular vampirism" = penetration of the prey surface and sucking the contents via specialised organelles) to myzocytotic extracellular parasitism, and finally to intracellular parasitism. The Apicomplexa demonstrate two main determinative evolutionary trends: i) the origination of epicellular parasitism on the apical surface of the host cell, observed mostly in gregarines and cryptosporidia, with significant modifications to the attachment apparatus and motility mode at the vegetative stage (trophozoite); and ii) origination of intracellular parasitism, typical of coccidia and Aconoidasida, accompanied by rejection of trophozoite polarity and motility. Early branching apicomplexans significantly differ from other Apicomplexa in that their trophozoites are equipped with an attachment apparatus that evolved at the apical end of the sporozoite (the first, invasive stage in the apicomplexan life cycle) and demonstrates an enormous diversity in architecture. In addition, their large trophozoites and gamonts are usually motile and, as our studies have shown, their locomotion differs from substrate-dependent, actin/myosin-based gliding motility described for the highly motile apicomplexan zoites. Basal apicomplexans appear

to use several motility mechanisms that correlate with various modifications of their cell cortex and represent specific adaptations to parasitism in different host body parts or organs.

In conclusion, I would like to point out that this thesis further emphasises the importance of research on apicomplexans occurring in different environments for a deeper understanding of the biology and evolutionary pathways of Apicomplexa. This is especially true for deep-branching apicomplexans, as they exhibit an enormous diversity in subcellular organisation, leading to the emergence of diverse parasitism strategies accompanied by highly specialised adaptations to their parasitic lifestyle. An intense research on invertebrate (especially marine) parasites is needed to establish a more realistic phylogenetic framework of Apicomplexa and to identify primitive and advanced parasitism strategies. Only the reconciliation of morphological and molecular phylogenetic data obtained from all apicomplexan lineages, including those to which no economic or medical significance is attributed, will allow reconstruction of pathways for the origin and evolution of parasitism in Apicomplexa.

As most of the enclosed publications present collective results summarised in multi-authored works, which arose on the basis of joint discussions and shared ideas, the following section is devoted to the specification of the applicant's (AV) contribution in terms of quality and content.

7.1 Valigurová A. (2012). Sophisticated adaptations of *Gregarina cuneata* (Apicomplexa) feeding stages for epicellular parasitism. PLoS One 7(8), e42606. AV contribution: 100% (holder of a research and bilateral project, author of the idea, conceptualisation of the study, design and implementation of experiments, manuscript preparation).

7.2 Valigurová A., Vaškovicová V., Musilová N., Schrével J. (2013). The enigma of eugregarine epicytic folds: where gliding motility originates? Frontiers in Zoology 10, 57. AV contribution: about 70% (holder of a research and bilateral project, conceptualisation and study design [contributed also by JS], material collection, performing experiments and microscopic analyses, data evaluation [except for freeze etching performed by NV], manuscript preparation, first and corresponding author).

7.3 Melicherová J., Ilgová J., Kváč M., Sak B., Koudela B., Valigurová A. (2014). Life cycle of *Cryptosporidium muris* in two rodents with different responses to parasitization. Parasitology 141(2), 287-303. AV contribution: about 50% (research project holder, significant contribution to the conceptualisation and study design, directing the study, contribution to data collection, significant contribution to data interpretation and manuscript preparation, corresponding author, supervisor of JM and JI).

7.4 Valigurová A., Paskerova G.G., Diakin A., Kováčiková M., Simdyanov T.G. (2015). Protococcidian *Eleutheroschizon duboscqi*, an unusual apicomplexan interconnecting gregarines and cryptosporidia. PLoS One 10(4), e0125063. AV contribution: about 65% (author of the idea, conceptualisation and study design, directing the study, participation in field sampling and material collection, data collection and analyses [also contributed by co-authors], conducting experiments and confocal microscopic analysis, manuscript preparation, first and corresponding author, supervisor of MK and AD).

7.5 Melicherová J., Mazourová V., Valigurová A. (2016). In vitro excystation of *Cryptosporidium muris* oocysts and viability of released sporozoites in different incubation media. Parasitology Research 115(3), 1113-1121. AV contribution: about 40% (bilateral project holder, significant contribution to the conceptualisation and study design, directing the study, contribution to the data interpretation and manuscript preparation, supervisor of JM and VM).

7.6 Diakin A., Paskerova G.G., Simdyanov T.G., Aleoshin V.V., Valigurová A. (2016). Morphology and molecular phylogeny of coelomic gregarines (Apicomplexa) with different types of motility: *Urospora ovalis* and *U. travisiae* from the polychaete *Travisia forbesii*. Protist 167(3), 279-301. AV contribution: about 30% (contribution to the material collection and processing, contribution to the microscopic observations and data interpretation, significant contribution to the manuscript preparation, supervisor of AD).

7.7 Schrével J., Valigurová A., Prensier G., Chambouvet A., Florent I., Guillou L. (2016). Ultrastructure of *Selenidium pendula*, the type species of archigregarines, and phylogenetic relations to other marine Apicomplexa. Protist 167(4), 339-368. AV contribution: about 25% (bilateral projects holder, contribution to the study design, participation in field sampling and material collection, significant contribution to

the electron microscopic data collection and interpretation, contribution to the manuscript preparation).

7.8 Chambouvet A., Valigurová A., Pinheiro L.M., Richards T.A., Jirků M. (2016). *Nematopsis temporariae* (Gregarinasina, Apicomplexa, Alveolata) is an intracellular infectious agent of tadpole livers. Environmental Microbiology Reports 8(5), 675-679. AV contribution: about 20% (participation in field sampling and material collection, collection and interpretation of electron microscopic data, contribution to the light microscopic observations, contribution to the manuscript preparation).

7.9 Diakin A., Wakeman K.C., Valigurová A. (2017). Description of *Ganymedes yurii* sp. n. (Ganymedidae), a new gregarine species from the Antarctic amphipod *Gondogeneia* sp. (Crustacea). Journal of Eukaryotic Microbiology 64(1), 56-66. AV contribution: about 30% (contribution to the study design, contribution to the data collection and interpretation, substantial contribution to the manuscript preparation, supervisor of AD).

7.10 Kováčiková M., Simdyanov T.G., Diakin A., Valigurová A. (2017). Structures related to attachment and motility in the marine eugregarine *Cephaloidophora* cf. *communis* (Apicomplexa). European Journal of Protistology 59, 1-13. AV contribution: about 40% (conceptualisation and study design, directing the study, significant contribution to the material collection and processing, contribution to the data collection and interpretation, contribution to the manuscript preparation, supervisor of MK).

7.11 Valigurová A., Vaškovicová N., Diakin A., Paskerova G.G., Simdyanov T.G., Kováčiková M. (2017). Motility in blastogregarines (Apicomplexa): Native and drug-induced organisation of *Siedleckia nematoides* cytoskeletal elements. PLoS One 12(6), e0179709. AV contribution: about 60% (author of the idea, conceptualisation and study design, directing the study, participation in field sampling, managing and performing material collection/processing, conducting *in vitro* experiments, data collection and analyses, manuscript preparation, first and corresponding author, supervisor of MK and AD).

7.12 Melicherová J., Hofmannová L., Valigurová A. (2018). Response of cell lines to actual and simulated inoculation with *Cryptosporidium proliferans*. European Journal of Protistology 62, 101-121. AV contribution: about 50% (research project holder,

author of the idea, conceptualisation and study design, directing the study, significant contribution to the data interpretation and to the preparation of the first manuscript draft, reworking the manuscript for submission, corresponding author, supervisor of JM).

7.13 Valigurová A., Pecková R., Doležal K., Sak B., Květoňová D., Kváč M., Nurcahyo W., Foitová I. (2018). Limitations in the screening of potentially anti-cryptosporidial agents using laboratory rodents with gastric cryptosporidiosis. Folia Parasitologica 65, 010. AV contribution: about 30% (contribution to the conceptualisation and study design, participation in material processing, conducting the histopathological analyses and interpretation of the microscopic data, manuscript preparation, first author).

7.14 Kováčiková M., Vaškovicová N., Nebesářová J., Valigurová A. (2018). Effect of jasplakinolide and cytochalasin D on cortical elements involved in the gliding motility of the eugregarine *Gregarina garnhami* (Apicomplexa). European Journal of Protistology 66, 97-114. AV contribution: about 40% (bilateral project holder, conceptualisation and study design, directing the study, significant contribution to the data collection and interpretation, contribution to the manuscript preparation, supervisor of MK).

7.15 Simdyanov T.G., Paskerova G.G., Valigurová A., Diakin A., Kováčiková M., Schrével J., Guillou L., Dobrovolskij A.A., Aleoshin V.V. (2018). First ultrastructural and molecular phylogenetic evidence from the blastogregarines, an early branching lineage of plesiomorphic Apicomplexa. Protist 169(5), 697-726. AV contribution: about 10% (bilateral project holder, participation in field sampling and material collection, contribution to the microscopic observations and to the manuscript preparation, supervisor of AD and MK).

7.16 Paskerova G.G., Miroliubova T.S., Diakin A., Kováčiková M., Valigurová A., Guillou L., Aleoshin V.V., Simdyanov T.G. (2018). Fine structure and molecular phylogenetic position of two marine gregarines, *Selenidium pygospionis* sp. n. and *S. pherusae* sp. n., with notes on the phylogeny of Archigregarinida (Apicomplexa). Protist 169(6), 826-852. AV contribution: about 10% (participation in field sampling and material collection, contribution to the microscopic observations and to the manuscript preparation, supervisor of AD and MK).