3. SUMMARY

The text above refers about the majority of characterized trematode peptidases; the fundamental enzymes for trematode existence, which are integrated in many physiological processes like pathogenesis, tissue invasion/migration, nutrition, immune evasion and host-parasite interactions.

In the history (until 1996), the peptidase catalytic activities in trematode extracts have been monitored. During 1980s and 1990s, the information of first cloned trematode peptidase genes were published and during last three decades cca 90 trematode peptidase sequences belonging to 19 peptidase families of 5 clans have been identified.

The most studied trematode peptidases have been of *Schistosoma mansoni* origin: the serine peptidase - cercarial elastase (of cercariae), cysteine peptidases - cathepsins B, L, F, C plus the asparaginyl endopeptidase SmAE and the aspartic peptidase - cathepsin D (of adult worms and some other life stages).

The recent computational cluster analysis revealed that the sequence *S. mansoni* elastase (the main cercarial penetration enzyme) is quite divergent from other serine peptidases of the S1 family. Cercarial elastase gene was proved in *S. mansoni, S. haematobium* and *Schistosomatium douthitti*, but not in the related *S. japonicum*. Mass spectrometry analysis confirmed cercarial elastase as an abundant enzyme in *S. mansoni,* whereas no cercarial elastase was found in *S. japonicum* or in the bird schistosomes *Trichobilharzia regenti* and *T. szidati*. Cercariae of these last three species probably use other peptidases for penetration; based on our results we suggest that at least in bird schistosomes these may be cysteine peptidases of the papain-like family (cathepsins B).

Papain-like peptidases (cathepsins) were found in 11 trematode species. The majority of papain-like peptidases was described as essential enzymes for nutrition (blood digestion) in adult worms. *Schistosoma mansoni* blood digesting peptidase cathepsin B1 was the first trematode peptidase cloned, whereas a related *S. mansoni* cathepsin B2 was identified quite recently (5 years ago). Successively, cathepsins B1/B2 of other trematode species have been characterized and localized (e.g. *S. japonicum, Fasciola hepatica, Clonorchis sinensis, Paragonimus westermani* and *T. regenti*/*T. szidati*). The necessity of cathepsins B for proper development of the flukes was proved by, e.g., *in vivo* biolistic analysis and knocking-down cathepsin B expression in *S. mansoni* adults. Therefore, cathepsins B might be targeted for design of novel schistosomiasis or general anti-

trematode inhibitor chemotherapeutics (e.g. K11777). *Schistosoma mansoni* cathepsin L was localized in the reproductive system and it is probably not involved in blood digestion. On the other hand, *F. hepatica*/*F. gigantica* cathepsins L1/L2 and cathepsin L of *P. westermani* probably represent major blood digestive peptidases localized in the gut of these worm species. *F. hepatica* cathepsins L1/L2 have already been employed in vaccine trials against cattle and sheep fascioliasis and a high protection level (72 - 79 % decrease in worm burden) was reached. Potential cathepsin L activity was detected also in cercarial extracts or in juvenile fluke extracts of our trematode models *T. regenti*, *T. szidati* and *Fascioloides magna*. The attempts to obtain sequences and to clone cathepsin L genes of these species are in progress. However, it is not possible reliably differentiate the activity of cathepsin F from the activity of cathepsin L with fluorogenic peptide substrates. Therefore, the noticed activity in *T. regenti*, *T. szidati* and *F. magna* protein extracts might originate from both cathepsins L and F.

Schistosome and clonorchid cathepsins F (*S. mansoni*, *S. japonicum* and *Clonorchis sinensis*) share the same localization in the intestine. Their major role is to provide a tool for nutrient processing by adult worms.

Remaining trematode papain-like peptidases, cathepsins C and asparaginyl endopeptidases, were confirmed as essential factors for *trans*-processing of fundamental blood digestive peptidases in blood-feeding flukes – cathepsins B of *S. mansoni*, *S. japonicum*, *F. hepatica*, *F. gigantica* and *P. westermani*. During the first step the asparaginyl endopeptidase cleaves the main part of cathepsin B pro-region (except Val87-Glu88 doublet). The processing is subsequently finished by cathepsin C, cleaving the remaining amino acid doublet to fully processed active form of cathepsin B. *In vitro* "cross-" *trans*-activation of *S. mansoni* cathepsin B by *Ixodes ricinus* recombinant asparaginyl endopeptidases and final "cross-" processing by rat cathepsin C was recorded. It suggests evolutionary fixed universal peptidase-activating system.

The last prominent peptidase participating in blood digestion cascade is the aspartic peptidase cathepsin D. Cathepsins D of *S. mansoni*/S. *japonicum* are speculated to be the more important ones in the process of hemoglobin degradation than cathepsins B. *In vitro* cleavage of hemoglobin by recombinant schistosome cathepsins D and B results in hemoglobin fragments of different length. Putative cathepsins D of *T. regenti* and *T. szidati* were also obtained, but deduced amino acid sequences did not blast significantly with known trematode cathepsin D sequences.

Four novel peptidase sequences of our two model organisms (*Trichobilharzia regenti* and *T. szidati*) were obtained and multiple biochemical characteristics of these peptidases were described. Our results were continualy compared with data on the best described trematode species - *Schistosoma mansoni*. It was shown that *T. regenti*/*T. szidati* cathepsins B1 and B2 sequences are similar to *S. mansoni* cathepsins B1/B2 by 77 % and 88 %, respectively. It evidences that bird schistosomes are appropriate comparative models for human *S. mansoni*. However, significant differences in the proteolytic equipment between *S. mansoni* and bird schistosomes have been revealed during our studies, showing that the flukes within one family may use different enzymatic tools during penetration of the host skin.

I believe that this work summarizing data on trematode peptidases can help to better understand multiple peptidase functions in trematode biology, as well as elucidate some novel aspects of parasite-host interactions based on proteolysis.