Comparative regeneration and phylogeny in adiaphanidan flatworms

PhD candidate: Alexandra Grosbusch
Supervisor: Priv.-Doz. Dr. Bernhard Egger
Institute of Zoology, University of Innsbruck

Introduction

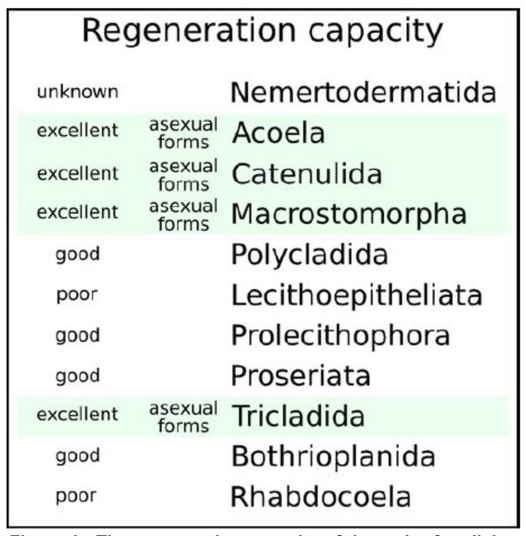

Figure 1: The regeneration capacity of the major free-living flatworm taxa.
In all groups with asexual reproduction, species with a pronounced regeneration capacity can be found (green shades). Not all species in the listed taxa are necessarily showing the same regeneration capacity; only a broad classification of regeneration capacities is given. [5]

Free-living flatworms were among the first animals where the extraordinary capabilities of regeneration were discovered. Since the beginning, most studies on regeneration have been done with representatives of the taxon Tricladida. They are as the champions of flatworm regeneration because they are able to regenerate a complete animal from a minute piece of tissue. This amazing phenomenon is enabled by a powerful stem cell system. A lot of studies have been done unraveling molecular pathways of regeneration but still many parts of the regeneration process are poorly understood [1]–[4]. However, free-living flatworms encompass a number of other taxa where regeneration has been observed, but for several orders of flatworms limited data on regeneration capabilities is available [5].

Comparison of regeneration processes in different flatworm taxa is necessary to determine whether regeneration is a conserved or a modified character in the large phylum Platyhelminthes. Such comparisons rely on a well resolved phylogenetic tree. However, the interrelationships of the free-living flatworms are not completely resolved, especially regarding the adiaphanidan clade [6]–[8].

Methodology

- **Sampling**

As Prolecithophora and Fecampiida are two poorly studied flatworm taxa, with no available laboratory models, they have to be collected from the field.

- **Determination**

Specimens will be determined by histological and molecular methods. Histological methods are necessary since few molecular data of the Prolecithophora and Fecampiida are available at this time and most of the prolecithophoran and fecampiid species are distinguished by characterisation of the pharynx and genital organs. Sequences of 18S and 28S ribosomal DNA will be determined to support the morphological species identification and to extend the currently available dataset.

- **Regeneration experiments**
  - *Amputations*

The presence or absence of regeneration abilities of prolecithophorans and fecampiids will be studied by performing amputations with as many species as possible. Live observations of the regenerates will be done to observe their outer shape and their behaviour.

  - *Fluorescent stainings*

Additionally, cellular events during regeneration will be visualised. Therefore, the stem cell system, the nervous system and the musculature will be monitored by fluorescent stainings

  - *Gene expression analysis*

We will analyse if head regeneration capacity can be stimulated by knocking-down beta-catenin in vivo in head-amputated prolecithophoran species, as seen in regeneration-deficient triclads.

- **Phylogenetic reconstruction**

As the phylogenetic position of the three taxa forming the Adiaphanida is not sufficiently resolved, I further investigate the interrelationships within this clade. A phylogeny of the Adiaphanida will be reconstructed using the two molecular markers (18S and 28S) and whole transcriptome datasets.

Figure 2: Sampling and laboratory
(A) and (B) Sample site in Croatia. (C) Sampling in the port of Punat. (D) and (E) Work in the laboratory in Punat.

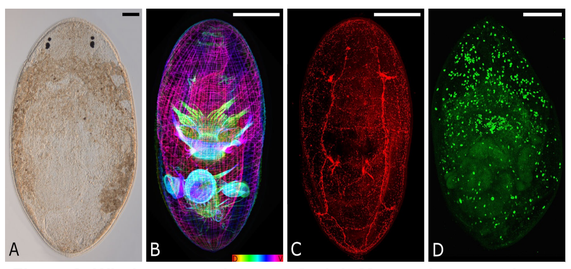

Figure 3: Whole mount pictures of adult *Monoophorum striatum* (Prolecithophora).
(A) Bright-field image of the four-eyed adult. (B) Confocal projection with depth coding of the musculature stained by phalloidin. (C) Confocal projection of the serotonergic nervous system. (D) S-phase cells (neoblast stem cells) labelled in green after 1 hour pulse. Scale bars: 100 µm.

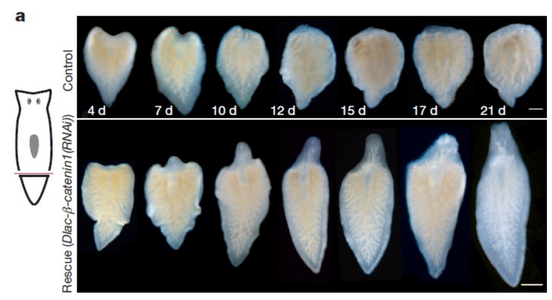

Figure 4: Rescue of the D. lacteum regeneration defect by b-catenin1 (RNAi).
a) Live images of a representative control (top) or b-catenin1(RNAi) tail piece (bottom) at indicated d.p.a. [9]

Outlook

As my work represents one of the first regeneration studies within this taxon, I will provide new data of principles of regeneration in free-living flatworm taxa and the interrelationships of the phylum Platyhelminthes will be further unravelled. Furthermore, understanding pattern formation in regeneration as well as the process of cell proliferation and differentiation in free-living flatworms will help to understand regeneration principles in more complex organisms.

References

[1] K. Agata, T. Tanaka, C. Kobayashi, K. Kato, and Y. Saitoh, “Intercalary regeneration in planarians,” *Dev. Dyn.*, vol. 226, no. 2, pp. 308–316, Jun. 2003.

[2] P. W. Reddien and A. S. Alvarado, “Fundamentals of Planarian Regeneration,” *Annu. Rev. Cell Dev. Biol.*, vol. 20, no. 1, pp. 725–757, Dec. 2004.

[3] S. A. Elliott and A. Sánchez Alvarado, “The history and enduring contributions of planarians to the study of animal regeneration,” *Wiley Interdiscip. Rev. Dev. Biol.*, vol. 2, no. 3, pp. 301–326, 2013.

[4] J. C. Rink, “Stem cell systems and regeneration in planaria,” *Dev. Genes Evol.*, vol. 223, no. 1–2, pp. 67–84, Mar. 2013.

[5] B. Egger, R. Gschwentner, and R. Rieger, “Free-living flatworms under the knife: past and present,” *Dev. Genes Evol.*, vol. 217, no. 2, pp. 89–104, Oct. 2007.

[6] B. Egger *et al.*, “A transcriptomic-phylogenomic analysis of the evolutionary relationships of flatworms,” *Curr. Biol.*, vol. 25, no. 10, pp. 1347–1353, Oct. 2015.

[7] C. E. Laumer, A. Hejnol, and G. Giribet, “Nuclear genomic signals of the ‘microturbellarian’ roots of platyhelminth evolutionary innovation,” *Elife*, vol. 4, 2015.

[8] C. E. Laumer and G. Giribet, “Phylogenetic relationships within Adiaphanida (phylum Platyhelminthes) and the status of the crustacean-parasitic genus *Genostoma*,” *Invertebr. Biol.*, vol. 136, no. 2, pp. 184–198, Jun. 2017.

[9] S.-Y. Liu *et al.*, “Reactivating head regrowth in a regeneration-deficient planarian species,” *Nature*, vol. 500, no. 7460, pp. 81–84, 2013.