

Transcriptome analysis on physiological roles in the mixed segment

P062

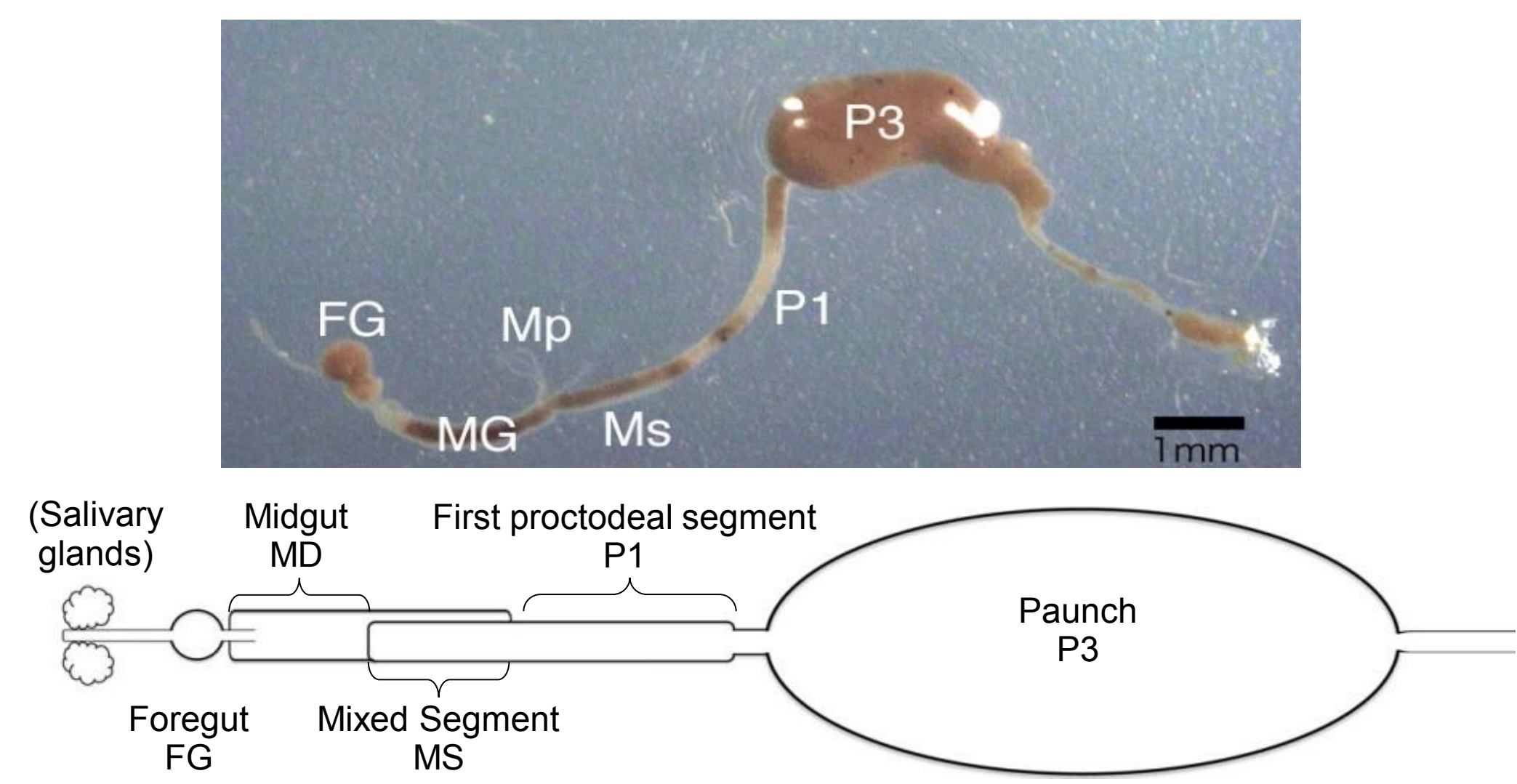
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1. Introduction

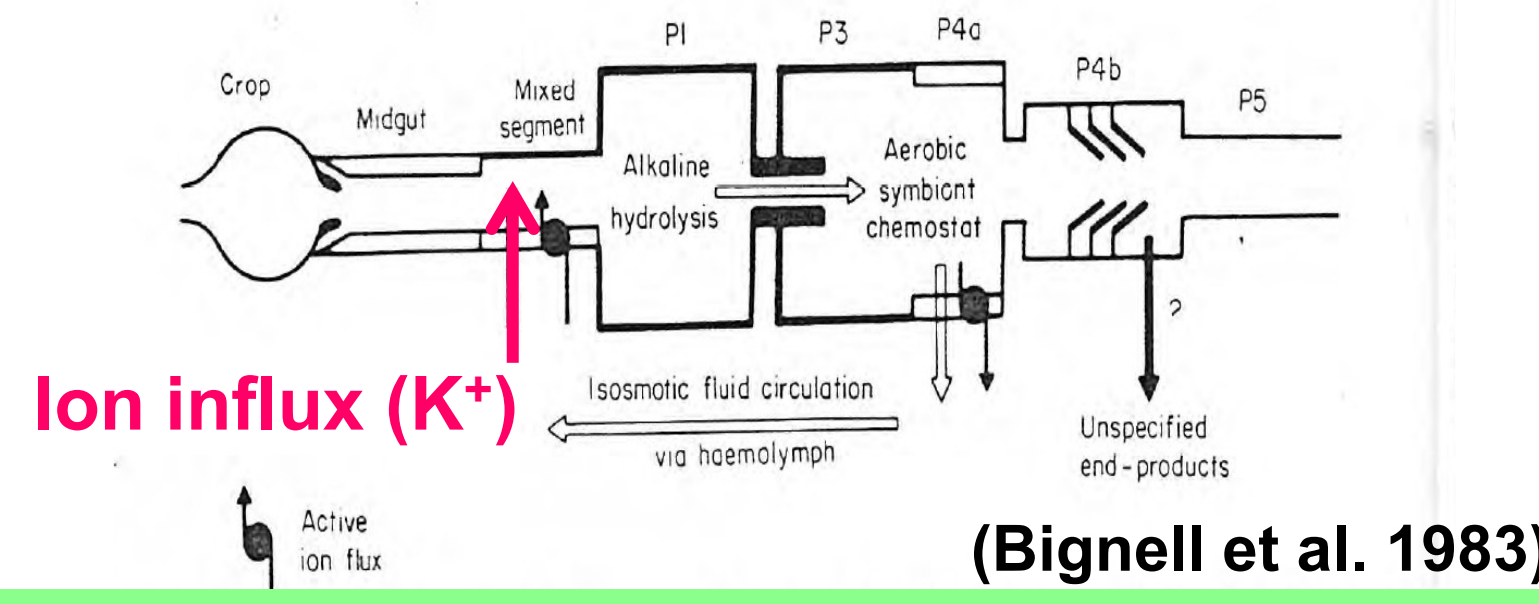
Termites comprise a group of social insects usually classified as a part of the order Blattodea (i.e. cockroaches) and are ecologically very important due to their ability of lignocellulose decomposition. Termites are divided into two groups namely, lower termites and higher termites (i.e. the family Termitidae). *Nasutitermes takasagoensis* is a wood-feeding higher termite. A remarkable feature of the gut in higher termites is the presence of so-called "mixed segment" between the midgut and first proctodeal segment (P1). The mixed segment consists of the mesenteric and proctodeal epithelial tissues. Although some physiological characteristics of the mixed segment have recently been clarified, yet the function has not been fully elucidated. Here, we carried out 454 pyrosequencing to profile the transcriptome of the mixed segment and compared with those of the midgut and P1 to understand the function of the mixed segment.

1.1 Digestive system in *N.takasagoensis*



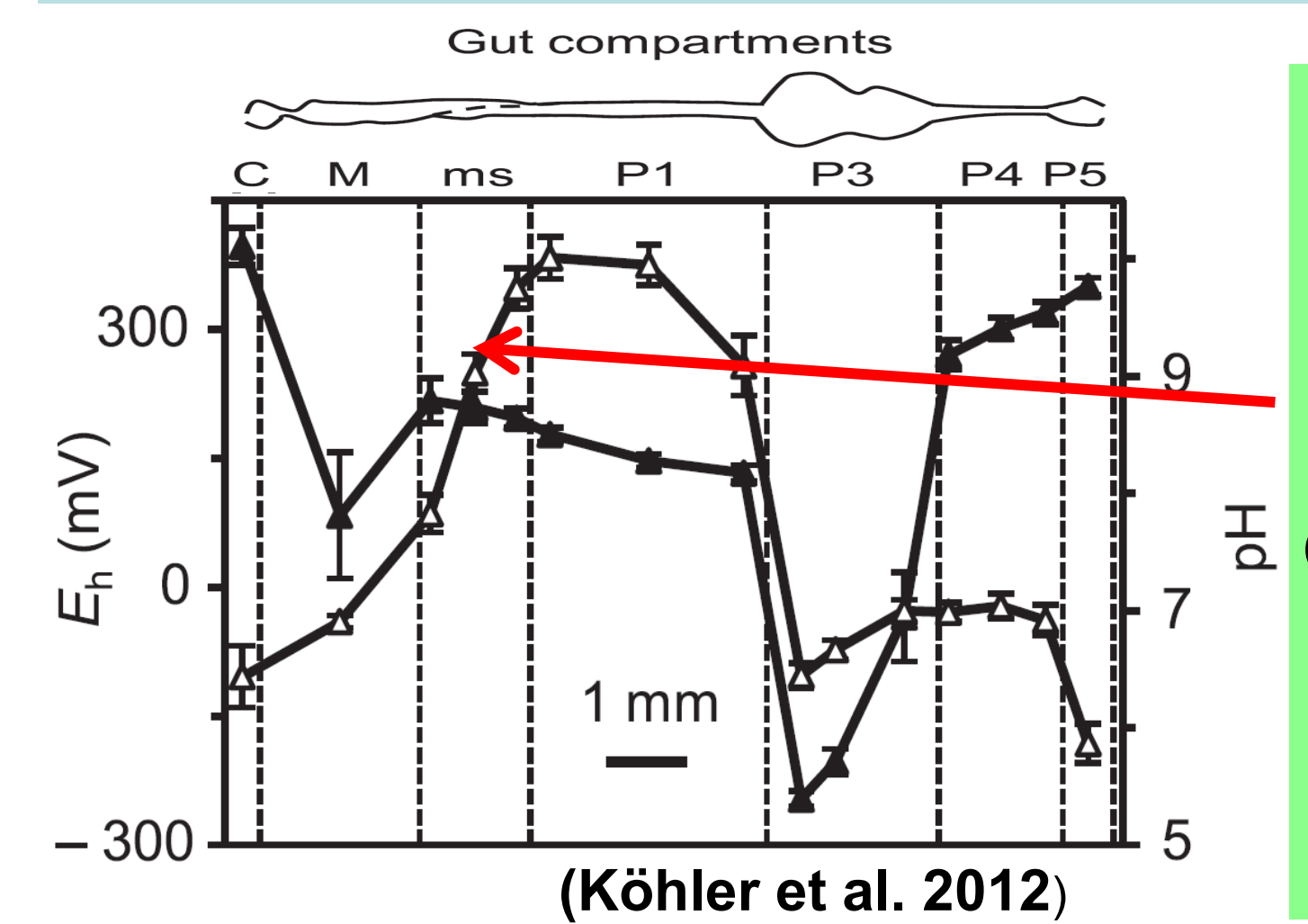
1.2 Previous findings

Model proposed by Bignell and co-workers



There is an active potassium ion flux across MS tissue to the lumen from haemolymph.

Axial profiles of pH along the gut of *N. corniger*, measured at the gut center.



pH sharply increased in the MS, and when it comes to P1, it shows the highest pH value.

What is function of the mixed segment??

2. Aim of the study

To obtain information on the genes and metabolic pathways working in the mixed segment

Transcriptome analysis

3. Experimental procedure

- Midgut (MD), mixed segment (MS), and P1 tissues were collected from worker termites
- cDNA libraries were prepared for the 454 GS junior sequencing
- Sequencing reads were assembled on Newbler ver. 2.7p
- Generated isotigs (transcripts) were annotated

3.1 Data annotation process

GO provides an ontology of defined terms representing gene products attributes across all species

dbCAN database describes the carbohydrate active enzymes

KEGG dealing with genomic information and metabolic pathways

Pfam is a database of protein families

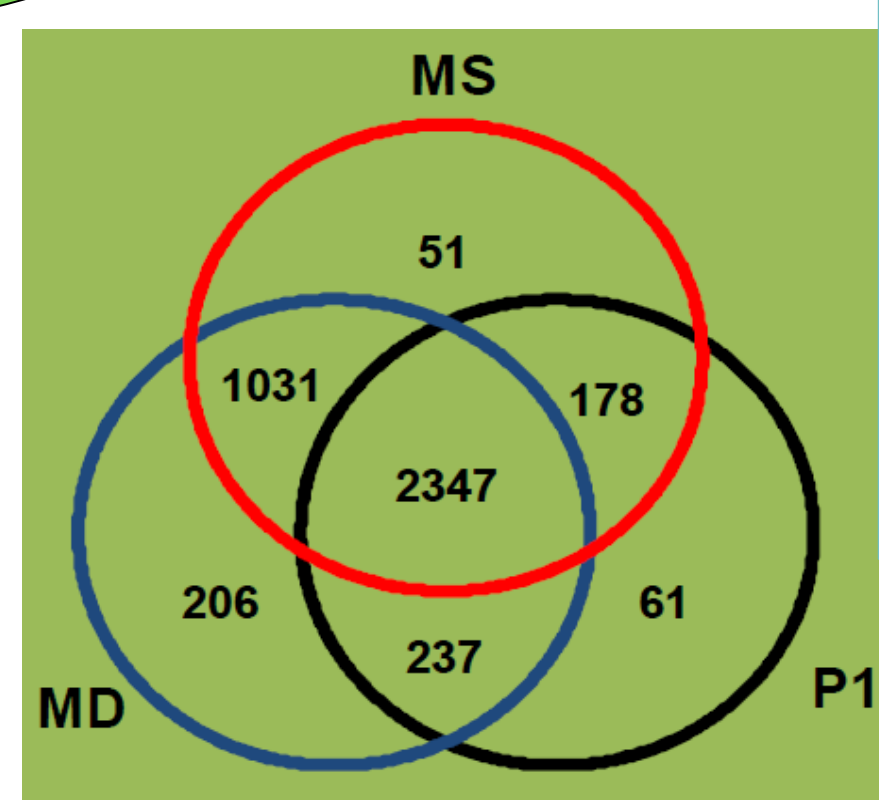
4. Results

4.1. Overall statistics

Description	MD	MS	P1
Putative host genomic mRNA	76197	45283	24603
Isotigs (transcripts)	4813	4563	3629
Isogroups (unigenes)	3821	3607	2823
Not considered for analysis	78517	70945	89610
Total number of reads	154714	116228	114213

4.2 Comparative analysis

Based on the present/absent information per gene for libraries



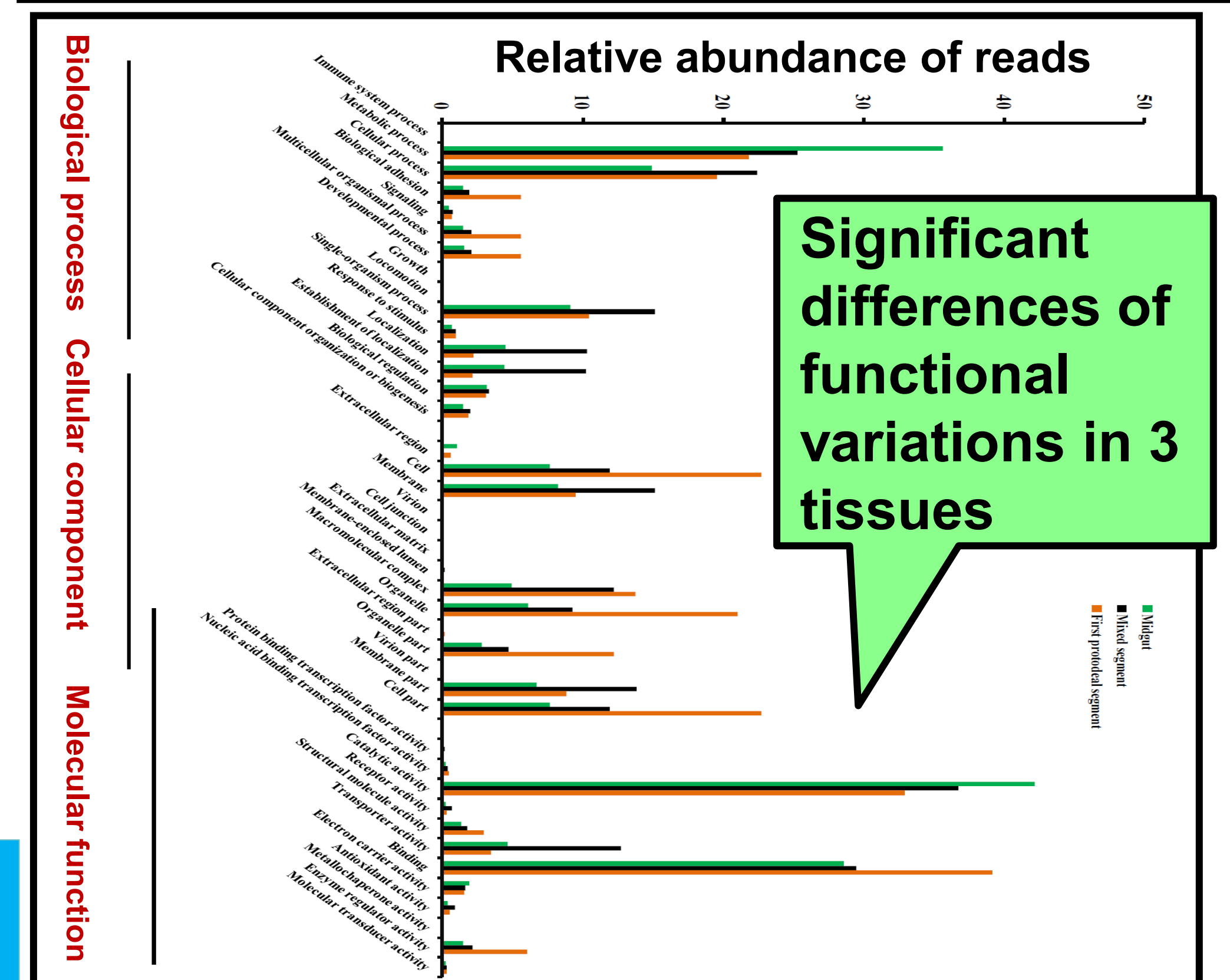
Comparative analysis and Pfam annotation revealed the differences in gene compositions in 3 tissues.

4.3 Pfam annotation

Highly expressed Pfam domains

- MD**
 - Haemolymph juvenile hormone binding protein
 - Insect allergen related repeat, nitrile-specifier detoxification
 - Glycosyl hydrolase family 9
 - Trypsin
- MS**
 - Ricin-type beta-trefoil lectin domain
 - ATP synthase subunit c
 - Actin
 - Several domains pertaining to V-ATPases
- P1**
 - Reverse transcriptase
 - Actin
 - Myosin head
 - Myosin tail

4.4 Level 2 Gene Ontology analysis



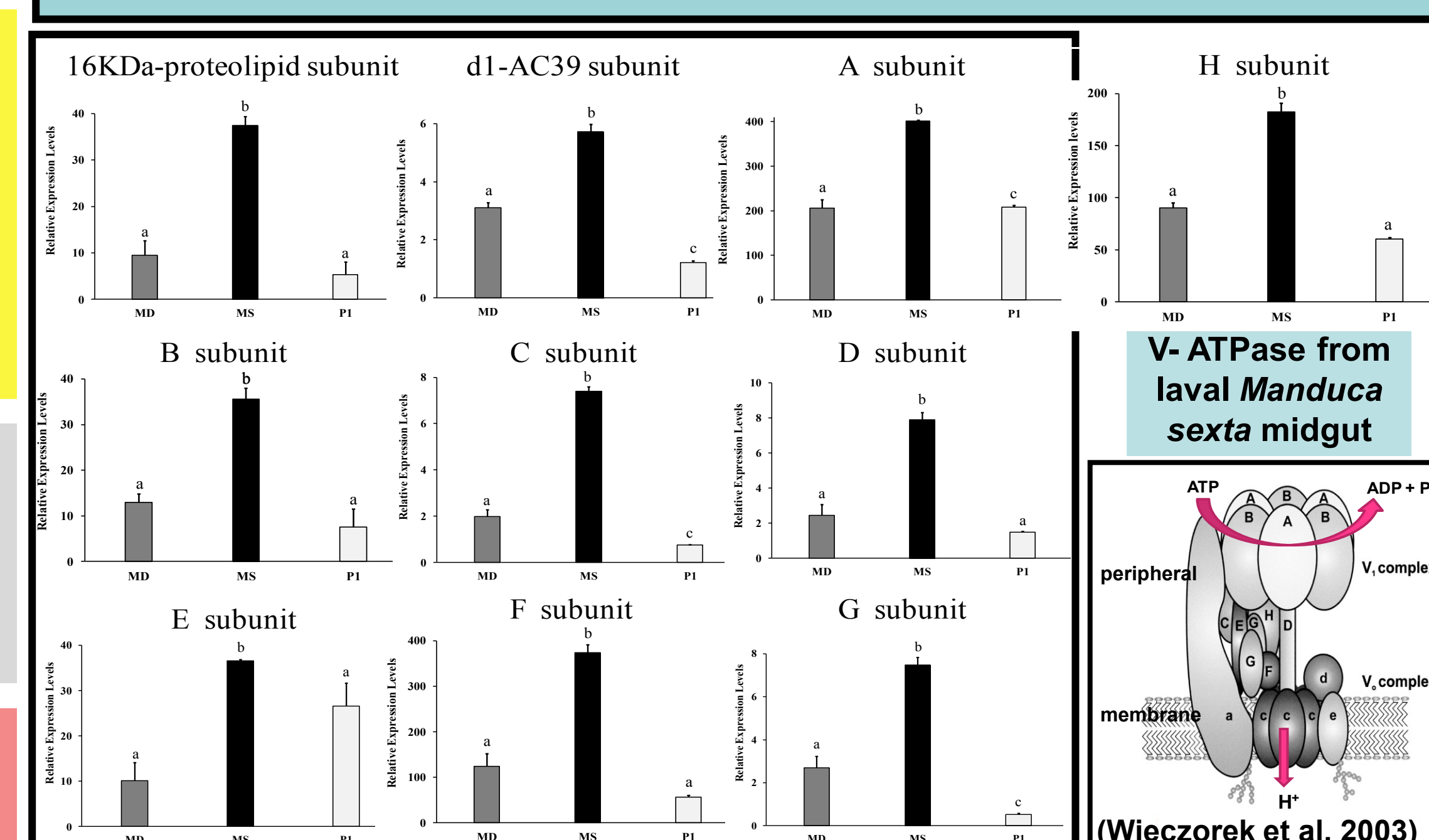
Significant differences of functional variations in 3 tissues

4.5 Highly abundant KEGG pathways identified in mixed segment (Relative abundance of reads)

- Glycolysis
- TCA cycle
- Oxidative phosphorylation
- Phagosome
- Lysosome
- Synaptic vesicle cycle

V-ATPases are involved in these pathways

4.6 Relative mRNA expression levels of V-ATPase subunits in 3 tissues



Dominant expressions of V-ATPase subunits in MS were confirmed with qPCR analyses.

We propose that V-ATPase genes play an important role in the mixed segment. Further, it may be responsible for an ion transport and catabolism