

# Comparative analysis of fatty acid profile in three eutardigrade species

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**Introduction:** Tardigrades colonize a wide range of habitats in which they can be predators, prey or primary consumers in food webs. Most species are herbivorous, feeding on cell fluid of algae and mosses, while others feed on bacteria, or prey on micrometazoans. The digestive system of tardigrades is divided into a foregut, a midgut, and a hindgut. The foregut mainly consists of a sucking buccal-pharyngeal apparatus, generally with a buccal tube that is narrow in herbivorous (Fig. 1) and wide in carnivorous (Fig. 2) species. Despite the wide range of food sources, details on food preference and on consequent lipid composition of tardigrade species are in practice unknown.

**Aim:** Comparative analysis of fatty acid composition of three eutardigrade species (and their substrates) belonging to different evolutionary lineages, also differing in colonized habitat and food sources.

**Species considered:** *Acutuncus antarcticus* (Hypsibiidae) is an Antarctic species inhabiting sediments of freshwater ponds. It is cultured in lab using the alga *Chlorococcum* sp. as food source. *Richtersius coronifer* and *Macrobiotus macrocalix* (Macrobiotidae) are terrestrial species colonizing the moss *Orthotrichum cupulatum* which is their food source.

**Methods:** Lipids were extracted with a chloroform/methanol method (Folch *et al.*, 1957). The total extracts were used to obtain the fatty acid methyl esters that were injected into a gas chromatograph. For each tardigrade species 10 replicates of 150-250 animals were used, while for moss and alga two replicates were utilized.

**Results:** In the three tardigrades species, the same 21 fatty acids belonging to saturated, monounsaturated (MUFA) and polyunsaturated (PUFA) groups were identified. Twenty-one fatty acids were detected in the moss *Orthotrichum cupulatum* colonized by *M. macrocalix* and *R. coronifer* live, and 19 fatty acids were identified in the alga *Chlorococcum* sp. in which *A. antarcticus* lives.

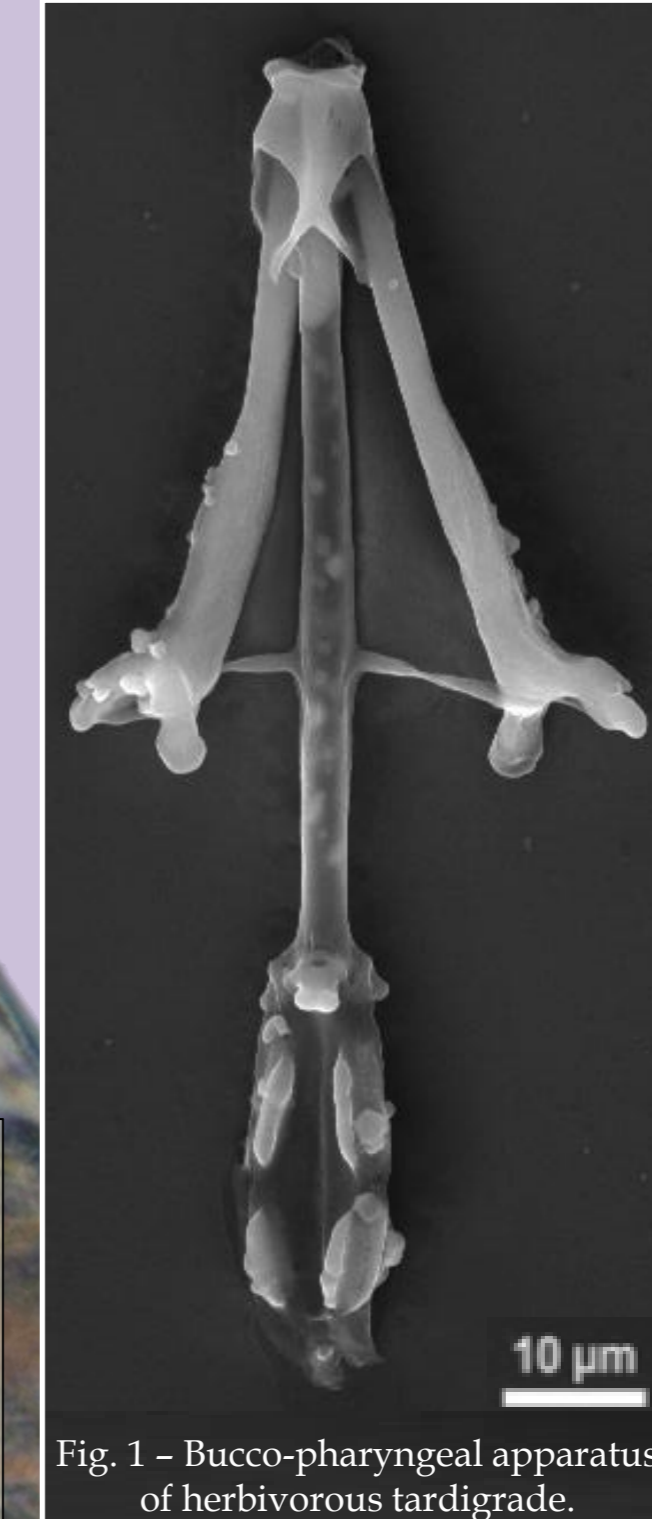
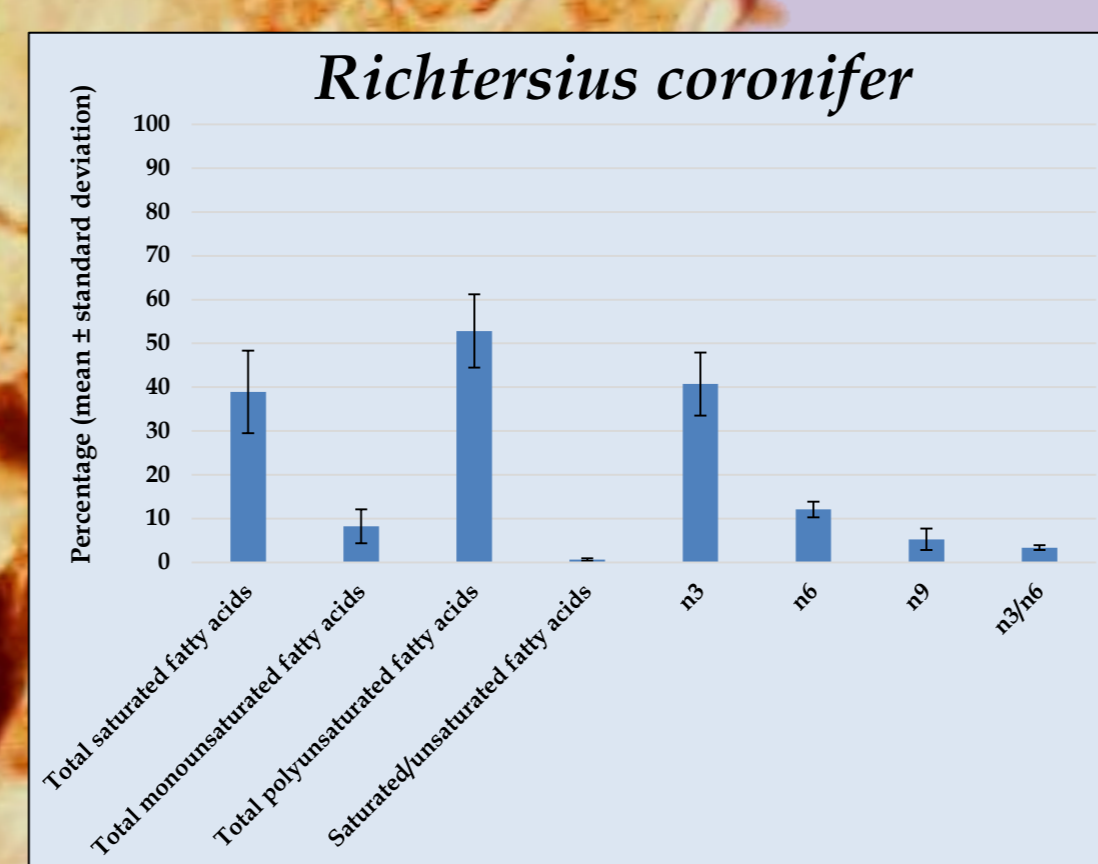
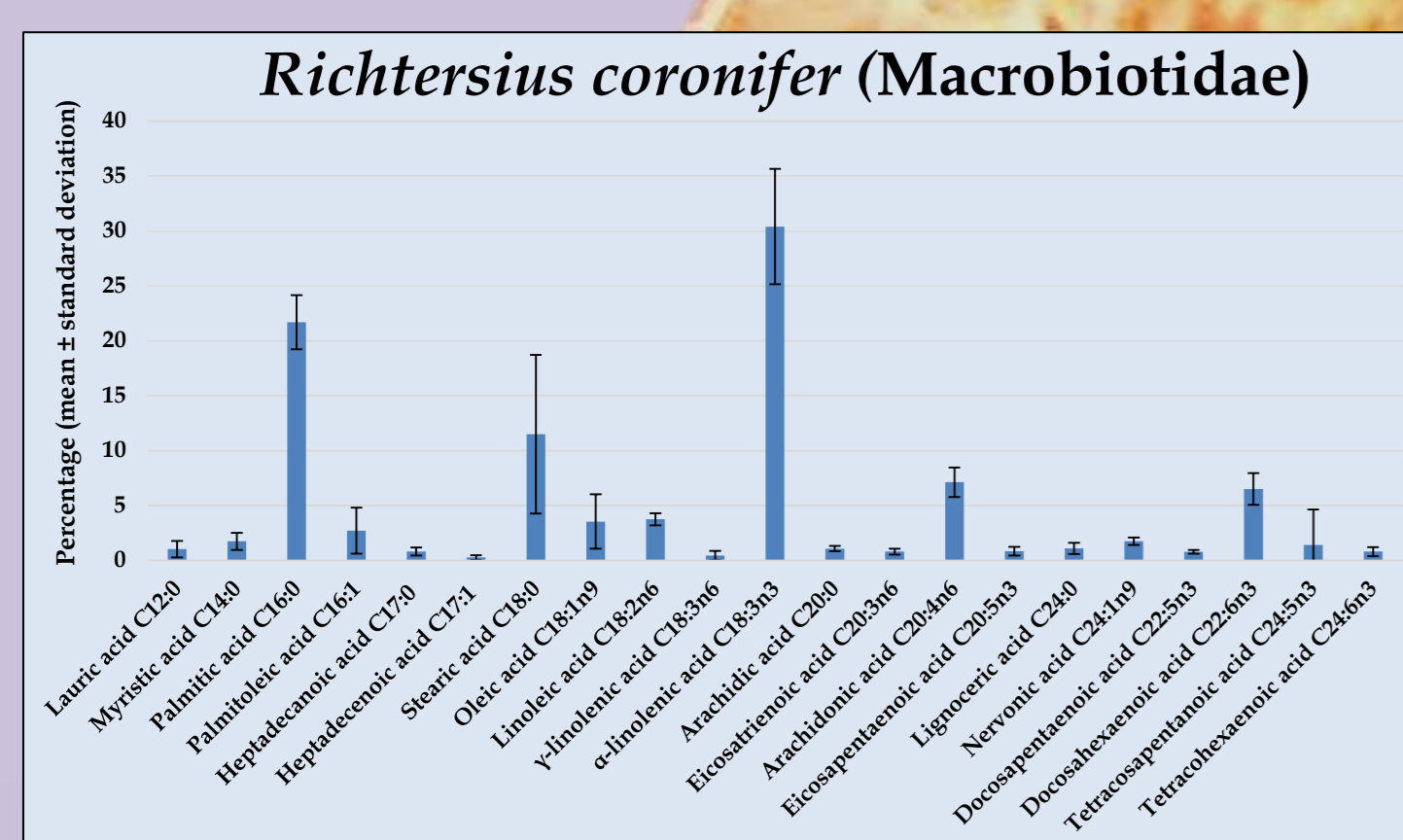


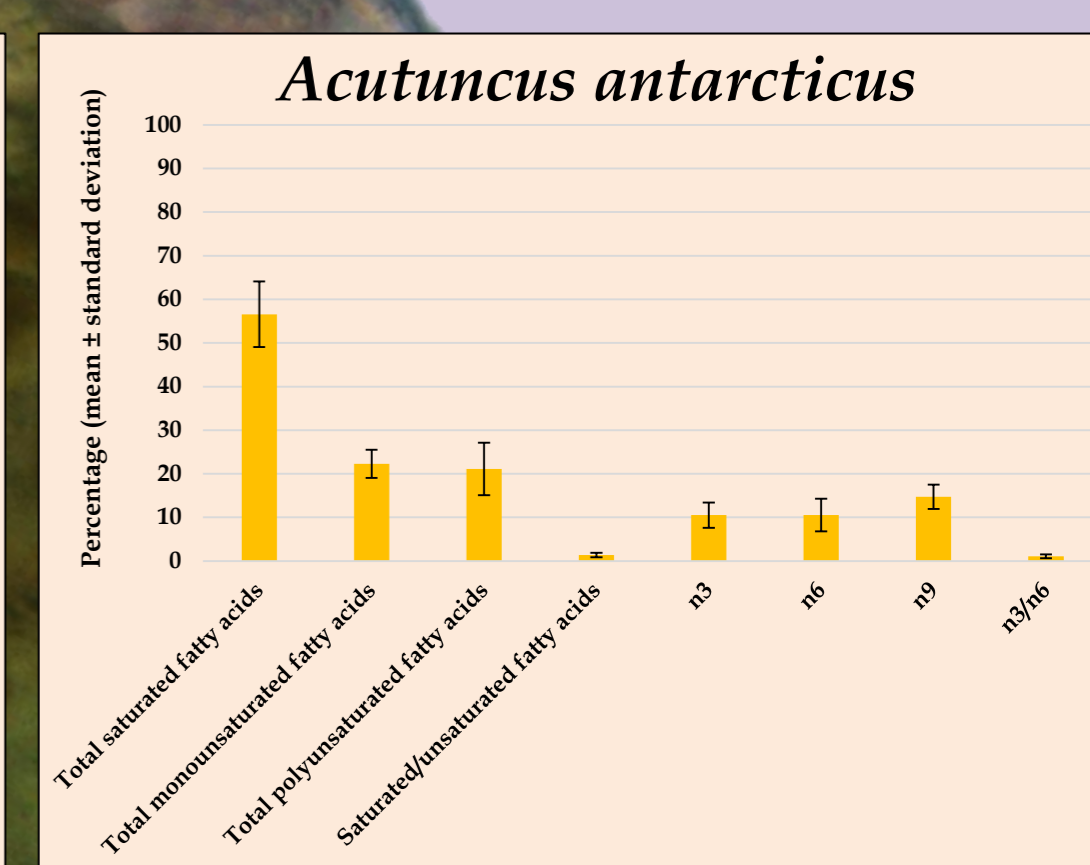
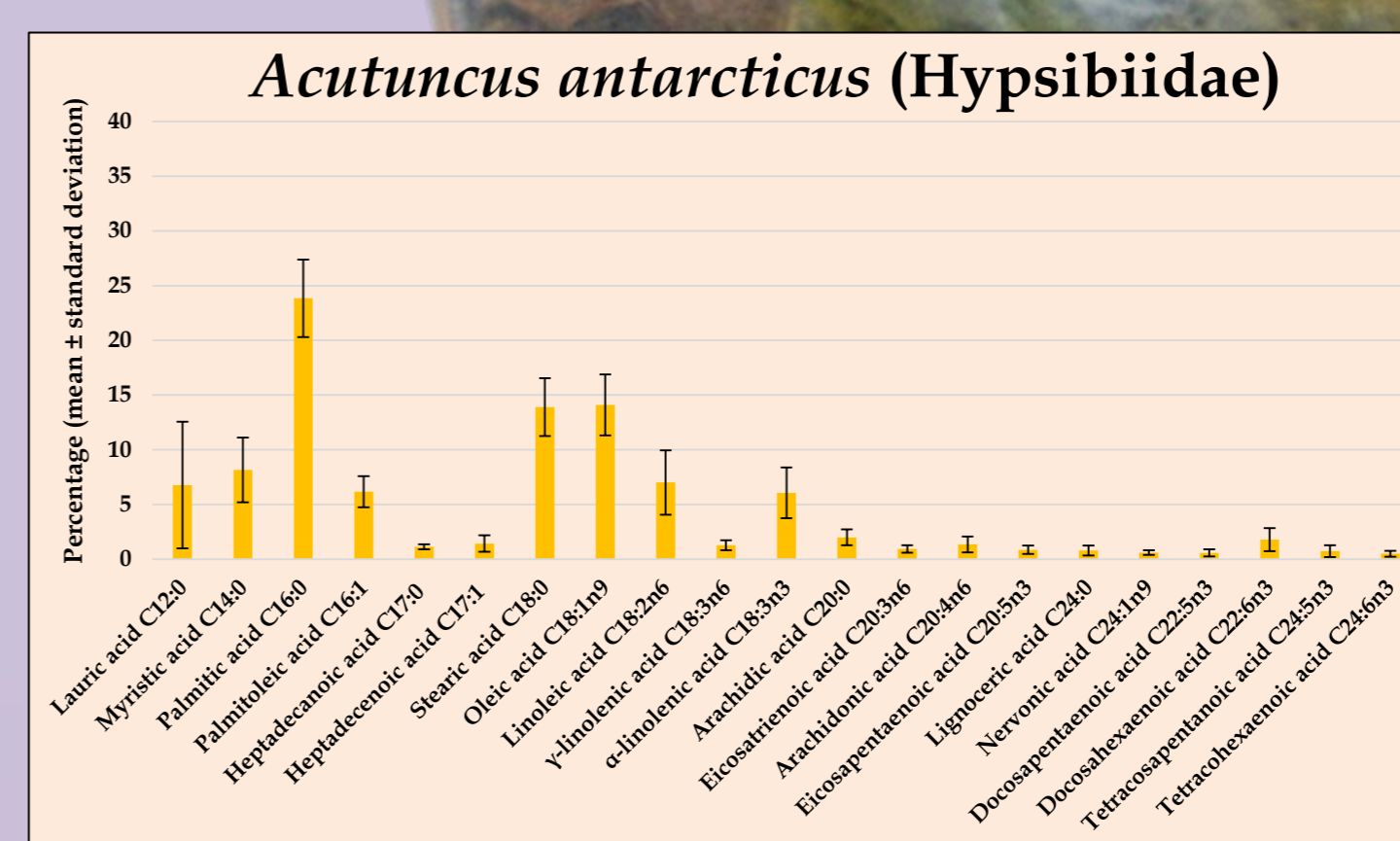
Fig. 1 - Bucco-pharyngeal apparatus of herbivorous tardigrade.



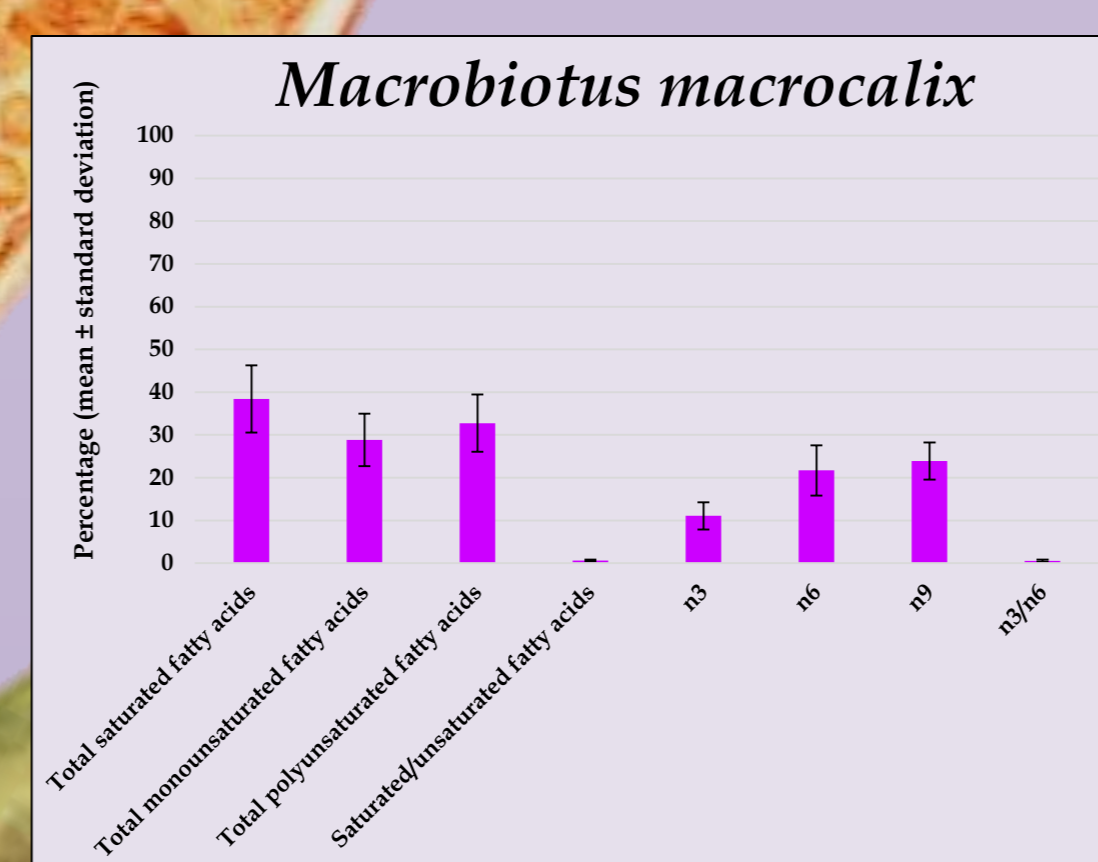
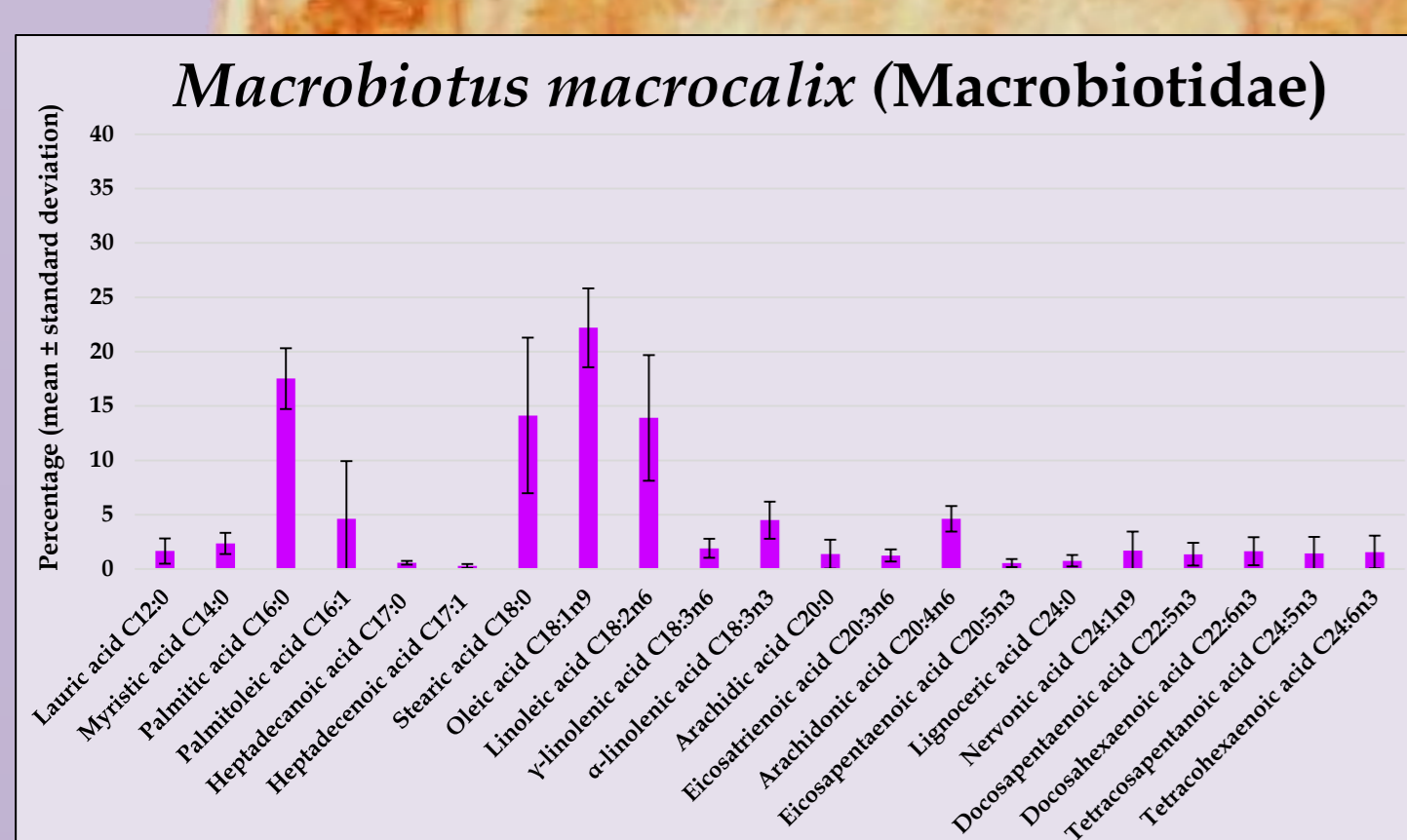
Fig. 2 - Bucco-pharyngeal apparatus of carnivorous tardigrade.



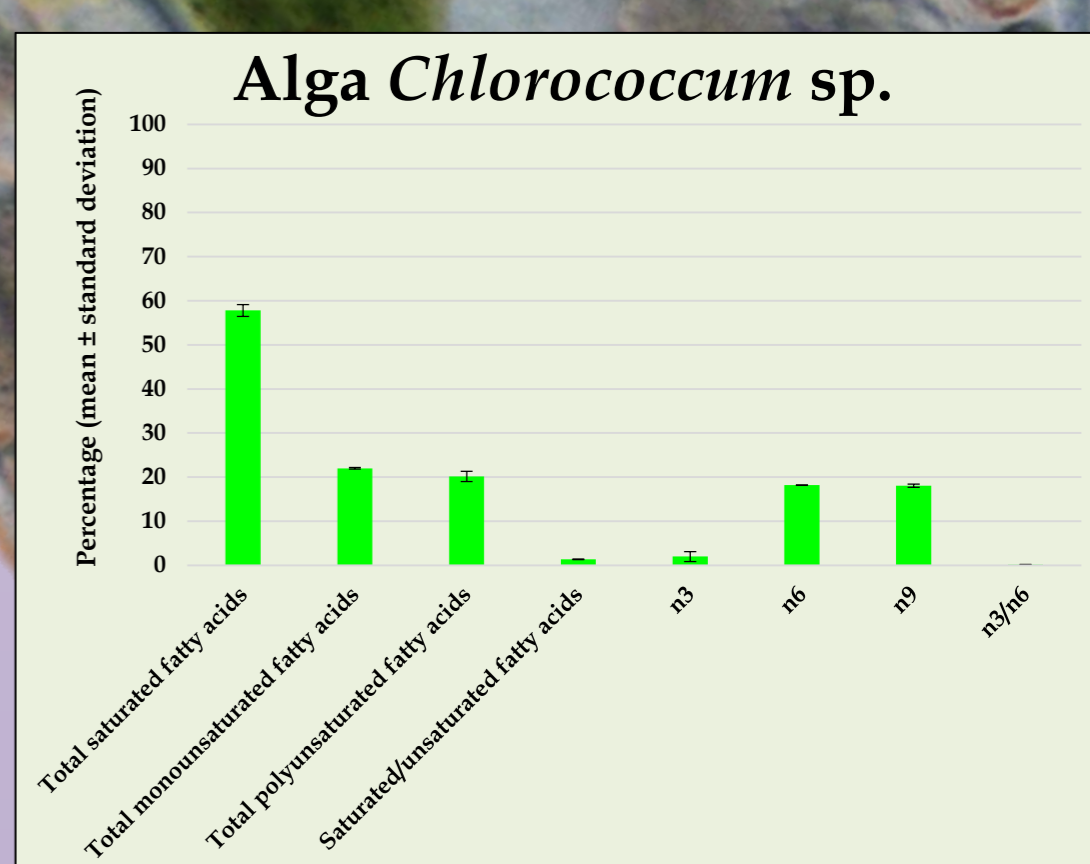
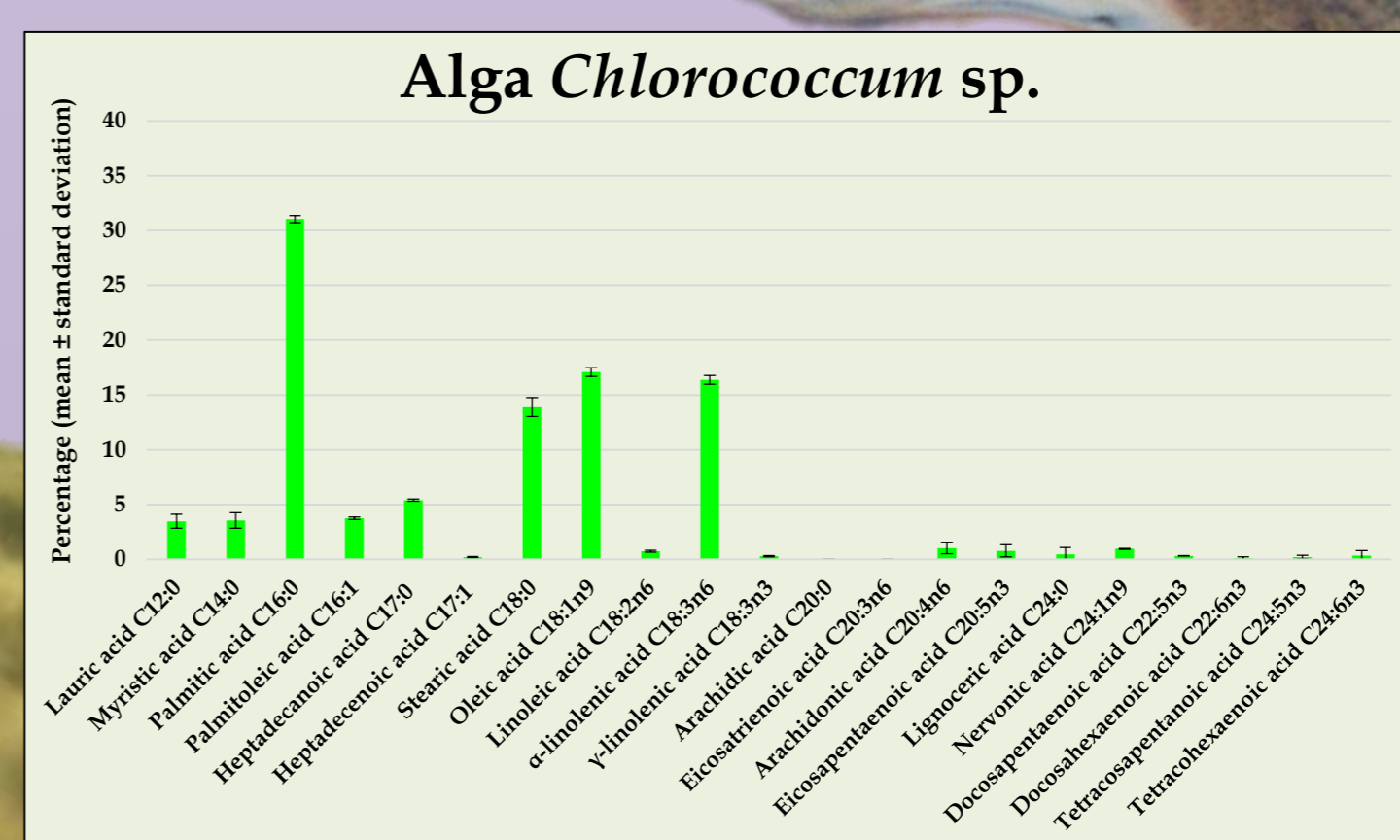
The most represented fatty acids were:  $\alpha$ -linolenic (C18:3n-3), palmitic (C16:0), stearic (C18:0), and arachidonic (C20:4n-6) acids. PUFA (52.8%) was higher than MUFA (8.2%) and higher than saturated fatty acids (38.9%).



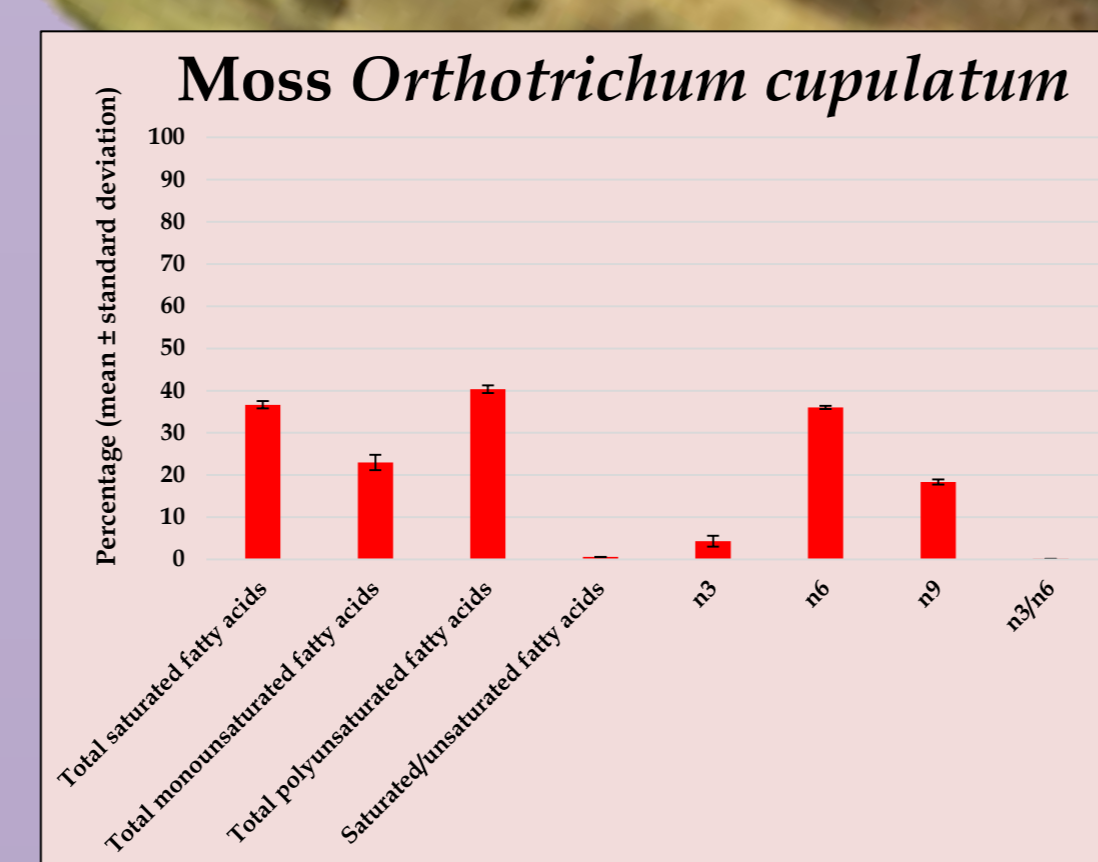
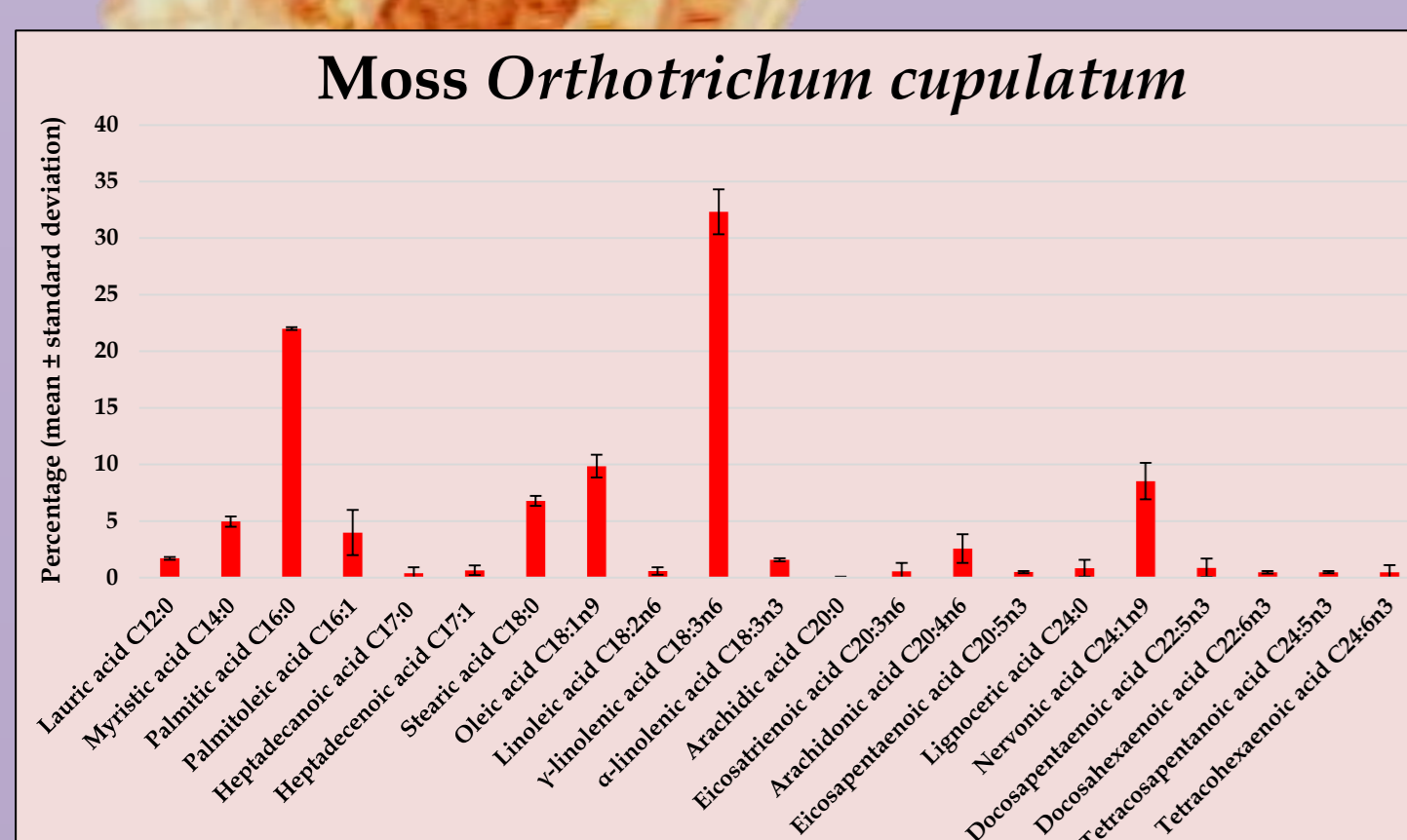
The most frequent fatty acids were: palmitic (C16:0), stearic (C18:0), oleic (C18:1n-9), and myristic (C14:0) acids. Saturated fatty acids (56.6%) were more abundant than MUFA (22.3%) and PUFA (21.1%).



The most represented fatty acids were: oleic (C18:1n-9), palmitic (C16:0), stearic (C18:0), and linoleic (C18:2n-6) acids. The saturated fatty acids (38.4%), MUFA (28.8%) and PUFA (32.8%) were uniformly distributed.



The most frequent fatty acids were: palmitic (C16:0), oleic (C18:1n-9),  $\gamma$ -linolenic (C18:3n-6) and stearic (C18:0) acids. Saturated fatty acids (57.8%) were more abundant than MUFA (22.0%) and PUFA (20.2%).



The most represented fatty acids were:  $\gamma$ -linolenic acid (C18:3n-6) and palmitic (C16:0) acids. The saturated fatty acids (36.7%), MUFA (23.0%) and PUFA (40.3%) were uniformly distributed.

**Discussion:** These data indicate clear differences in the fatty acid composition and amount among the three tardigrade species. The comparisons among fatty acid profiles of the tardigrade *M. macrocalix*, and *R. coronifer* and that of the moss *O. cupulatum* show that tardigrade species inhabiting the moss eat the moss cell content but use/transform the fatty acids in different ways, indicating different biochemical needs.

The fatty acids profile of *A. antarcticus* reflects the profile evidenced in its food source (alga *Chlorococcum* sp.), even if the high presence of saturated fatty acids is similar to the one evidenced in a previous study of the carnivorous tardigrade *Paramacrobiotus richtersi* (see Rizzo *et al.*, 2010).

**References:** Folch *et al.* 1957 - The Journal of Biological Chemistry, 226: 497-509; Rizzo *et al.* 2010 - Comparative Biochemistry and Physiology, 156: 115-121.

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