Analysis of the Composition of the Cuticula (Shell) of Isopods

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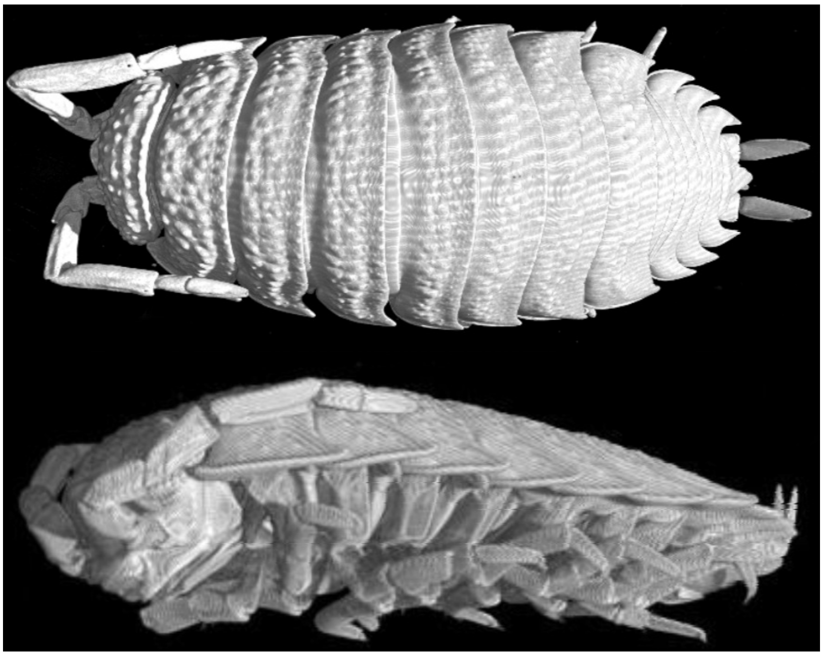

Fig. 1. Overview of *P. scaber* using SRµ-CT, top view (upper) and transversal from the side (lower). Only the mineralized parts (cuticula) of the animal are imaged because these have higher X-ray absorption.

The term "Isopoda" (woodlice and related species) describes an order of animals belonging to the Malacostraca class. The next higher classifications are the subphylum Crustacea and phylum Arthropoda, to which 80 % of all animal life forms belong, including insects, spiders, scorpions and millipedes. Arthropods live in almost all habitats, spanning from the poles to deserts, from the highest mountains to the deepest seas, and even underground or inside of other living creatures.

They first evolved in the Cambrian Period more than 500 million years ago. Over the course of time, a great variety of them have evolved, and some – like the trilobites – are also already extinct. Fossil records suggest that the Euthycarcinoid group of arthropods were the first terrestrial creatures.[1] 75 % of all Crustaceans are classified in the Malacostraca class. In addition to isopods, this class also counts crabs, lobsters and crawfish among its members. 3600 species of isopods are terrestrial and 5400 are aquatic.[2]

Figure 1 shows an image of the isopod *Porcellio scaber* taken using synchrotron radiation-based micro computer tomography (SRµCT). The image shows only the mineralized parts of the body. These have higher X-ray absorption than the soft tissue and can therefore be shown separately. In contrast to vertebrates, isopods have an exoskeleton, i.e. no internal bones (endoskeleton), but only a mineralized shell. That is why isopods play such an important role as a model organism in biomineralization; they build up minerals in their exoskeleton (also called the "cuticula").[3-5] The mineral portion consists of magnesium calcite, amorphous calcium carbonate (ACC)[6,7] and amorphous calcium phosphate (ACP). A part of the isopods' magnesium substitutes the calcium in the calcite; it is therefore magnesium calcite.[4] It is also presumed that the magnesium contributes to the kinetic stabilization

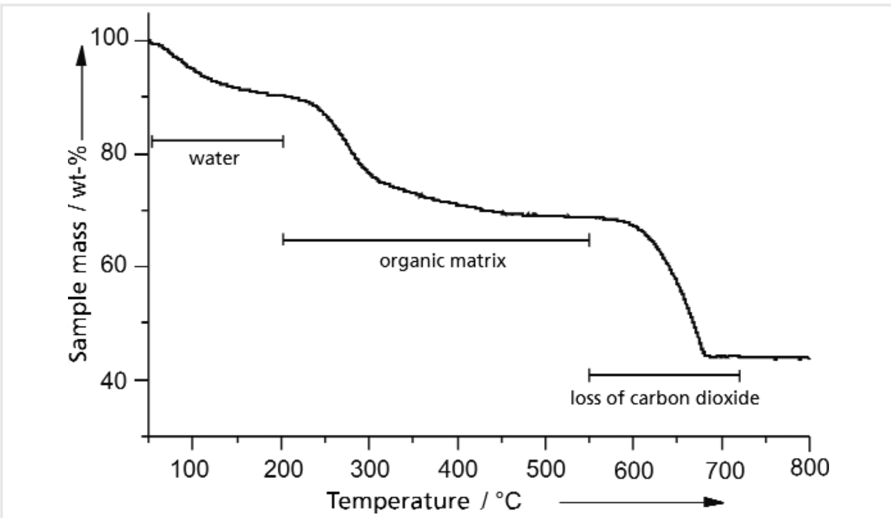

Fig. 2. Mass loss of the cuticula of *Philoscia muscorum*

Species	Ca	Mg	Water	Organic matrix	Entire mineral	$CaCO_3$	Mg-calcite	ACC
Philoscia muscorum[13]	25.4	0.49	10.5	21.2	68.3	56.0	17.5	38.5
Porcellio scaber[4]	24.3	0.71	8.0	24.8	67.2	49.6	14.5	35.0
Armadillidium vulgar[4]	29.8	0.72	9.7	11.7	78.6	64.8	10.8	54.0

Species	ACC/ calcit *w:w*	Mg/Ca *w:w*	$MgCO_3$ in Mg-calcit [mol%]	Mg in Mg-calcit	Mg not in Mg-calcit	Ca not in $CaCO_3$	ACP
Philoscia muscorum[13]	2.19	0.019	1.14	0.05	0.44	3.0	7.5
Porcellio scaber[4]	2.41	0.029	3.63	0.13	0.58	4.5	11.2

Tab. 1. Composition of the cuticula. Shown is the corresponding mass fraction *w* in weight-%, unless otherwise noted

(i.e. prevention of crystallization) of ACC.[8-10]

The cuticulae of different kinds of isopods were analyzed by means of quantitative powder diffraction (XRD), thermogravimetry (TG) and atomic absorption spectroscopy (AAS). Their compositions were then compared with the behavior and habitat of the respective creatures.[4,13] The cuticulae of the animals were collected by Dr. A. Ziegler (University of Ulm).

Presented here is the analysis with the example of *Philoscia muscorum*. Based on the powder diffractometric data, a Rietveld refinement was carried out which determined the proportion of crystalline magnesium calcite in the sample and the proportion of magnesium bound up in the Mg-calcite.

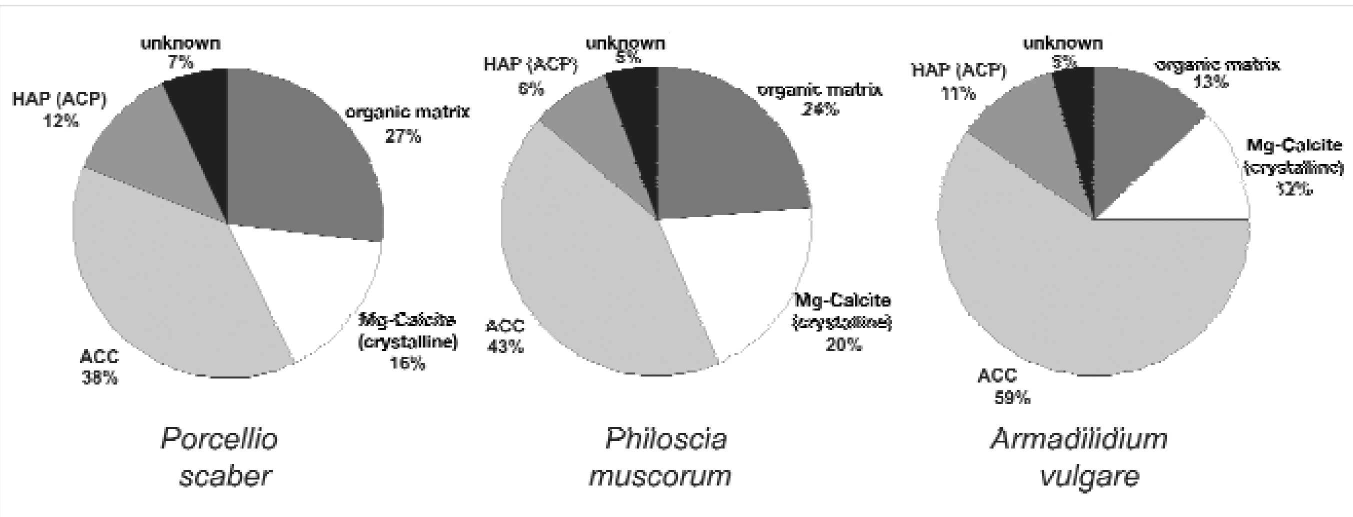

Fig. 3. Comparison of the cuticula composition of *Philoscia muscorum*, *Porcellio scaber* and *Armadillidium vulagare*

For the thermogravimetric analysis, the samples were heated at 3 K/min to 1000 °C in a dynamic oxygen atmosphere (figure 2). The evaporation of water (60 °C to 200 °C), the decomposition of the organic material (200 °C to 550 °C) and the decarboxylation of the carbonate (550 °C to 730 °C) can be seen.

During decomposition, mainly carbon dioxide and water are formed, and during decarboxylation, carbon dioxide. The resulting mass loss makes it possible to determine the amount of calcium carbonate in the sample. Since the amount of calcium carbonate is now known as a result of the thermogravimetric analysis, and the amount of magnesium calcite as a result of the quantitative powder diffraction, the amount of ACC can now also be determined.

Previous analyses have shown that not all of the calcium detected by means of AAS is bound up in the calcium carbonate.[4] By analyzing the diffraction pattern of residuals of the thermogravimetric analysis (1000 °C), it was shown that the cuticula also contains amorphous calcium phosphate (ACP) which crystallizes during heating in the TG. The function of calcium phosphate in the cuticula is not yet known. *In vitro* experiments under physiological conditions have demonstrated that small amounts of phosphate prevent the crystallization of calcite.[11,12] This may play a role in ACC formation and/or in improving the mechanical properties of the cuticula.

The results of the analysis are presented in table 1 and figure 3.

Additionally shown is the data for the species *Porcellio scaber* and *Armadillidium vulgare*. It can be seen that the compositions of the cuticulae of *Philoscia muscorum*[13] and *Porcellio scaber* are quite similar to each other. Both of these animals exhibit a similar tendency to flee from their natural enemies in self-protection. To be lighter and more flexible, their cuticulae are less mineralized than that of *Armadillidium vulgare*; this animal coils up in self-defense. Ten different kinds of isopods were analyzed and compared in the manner described.[13]

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