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Short Note

Dickdellia on *Colossendeis*: testing ectoparasitism with stable isotopes

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Introduction

Many pycnogonids are ectoparasites of benthic invertebrates. Even gastropods are on their menu (reviewed in Dietz *et al.* 2018). It is somewhat ironic that a marine gastropod, *Dickdellia labioflecta* (Dell, 1990), might have reversed this principle. Hedgpeth (1964), Sirenko (2000) and Schiaparelli *et al.* (2008) observed clutches of eggs and young *Dickdellia* snails on pycnogonids. Sirenko (2000) suggested that this might be a case of ectoparasitism and not just phoresy.

Lehmann *et al.* (2007) studied *Dickdellia* eggs and juveniles attached to a male *Colossendeis megalonyx* Hoek, 1881 and found sections of the sea spider's cuticle under larger *Dickdellia* individuals in which the distal cuticle layers were absent. These sections seemed to open up the interior of the pycnogonid, and thus were a potential channel of nutrition for the snails. These sections were absent under small embryos, indicating that they had been rasped by the developing snails. Lehmann *et al.* (2007) concluded that the snails might indeed be ectoparasites.

However, the ectoparasitism hypothesis needs more evidence to become a theory. We have therefore reanalysed the specimens and measured stable isotope contents of both snails and 'host' pycnogonids. Stable isotopes have been used to infer trophic levels for various marine invertebrates (Post 2002). We thus hypothesized that a parasitic relationship between *Dickdellia* and *Colossendeis* would leave different isotopic fingerprints from, for example, phoresy.

Materials and methods

We removed tissue samples from embryos and small and large juveniles of *Dickdellia* attached to the male *C. megalonyx* from Lehmann *et al.* (2007) (collection number ZSM Mol 20021231) and from an adult *Dickdellia* from the same sample event (collection number ZSM Mol 20020906). Moreover, we took samples from *C. megalonyx* cuticles and leg contents, again from the same specimen that had been the 'host' of the *Dickdellia* individuals. Results are given in δ notation relative to international standards ($\delta^{15}N$: AIR, $\delta^{13}C$: VPDB), where $\delta = (R_{sample} - R_{standard})/R_{standard}$ given in ‰. Isotope ratios (R) were determined with an isotope ratio mass spectrometer (Delta Plus, Thermo Finnigan) coupled with an elemental analyser (NC 2500, Carlo Erba) and calibrated with a laboratory standard (peptone) of known composition ($\delta^{15}N = 4.93$‰, $\delta^{13}C = -23.80$‰). Carbon and nitrogen contents were calculated from ratios of peak areas *vs* sample weights using standards (atropine, cyclohexanone 2,4-dinitrophenylhydrazone). A low-weight sample outside of the linearity range (two pooled embryos) was corrected with standards of similar weight. The precision (1 SD) of the isotope analyses was better than 0.2‰, except for the low-weight samples, for which 0.4‰ is a typical error.

Results and discussion

All results are given in Table I. We found almost the same $\delta^{15}N$ values for large juveniles and adult *Dickdellia* individuals (7.8–7.9‰), while the embryos had a slightly higher $\delta^{15}N$ value (8.1‰). Compared to this, the *Colossendeis* sample $\delta^{15}N$ values were 5.0‰ for the cuticle and 8.9‰ for the leg muscle, thus differing by almost 4‰. The $\delta^{15}N$ values of *Dickdellia*, at first sight, do not fit with the assumption of *Dickdellia* feeding on *Colossendeis*, given the frequently used trophic ^{15}N enrichment of ~3‰ of an animal relative to its food (Peterson & Fry 1987). However, the values make more sense if the life history of *Dickdellia* is considered. Lehmann *et al.* (2007) suggested that *Dickdellia*

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Table I. $\delta^{15}N$ and $\delta^{13}C$ values of *Dickdellia* and *Colossendeis* samples.

Sample	Amount (mg)	Peak area N (Vs)	Peak area C (Vs)	$\delta^{15}N$ *vs* AIR	$\delta^{13}C$ *vs* VPDB	%N	%C
C. m., cuticle first leg from egg clutch area	0.636	55.1	67.7	5.0	-27.2	11.6	45.6
C. m., first leg tissue	0.525	52.3	55.9	8.9	-26.0	13.3	45.6
D. l., adult	0.738	71.5	78.3	7.9	-24.7	13.0	45.4
D. l., large juvenile	0.505	46.1	43.4	7.9	-25.8	11.6	43.1
D. l., large juvenile	0.690	54.4	66.4	7.8	-24.9	10.6	41.2
D. l., small juveniles/embryos	0.053	3.3	3.0	8.1	-25.7	8.0	28.8
Mean value				8.0	-25.3		
SD				0.2	0.6		

C. m. = Colossendeis megalonyx, D. l. = Dickdellia labioflecta.

individuals might feed on the pycnogonids by rasping their cuticles and sucking out their body contents. Hence, their food would be a mix of both sources, and the expected $\delta^{15}N$ values of the hosts would be approximately the average of both tissues (i.e. ~7‰). For molluscs, Vanderklift & Ponsard (2003) have inferred a ^{15}N enrichment of only ~1‰ in the consumer relative to its food. This would mean that *Dickdellia* individuals feeding on both tissue types of *Colossendeis* would exhibit $\delta^{15}N$ values pf ~8‰, which corresponds to the values we found for large juveniles and the adult. These stages were taken from rasped cuticle areas, and the adult is supposed to show the same $\delta^{15}N$ value, as it should have had the same feeding type as the large juveniles. Conversely, the small juveniles or embryos have been taken from an unrasped cuticle section; in other words, they were not yet consuming a pycnogonid diet. Instead, they should have had a lecithotrophic past within the eggshell, and their $\delta^{15}N$ value should be that of a *Dickdellia* consumer (i.e. ~8.8‰), which fits within analytical error (2 s = 0.8‰, low-weight sample). Various isotope ratios of host tissue and blood have been recorded for other ectoparasite–host associations as well (Demopoulos & Sikkel 2015).

The $\delta^{13}C$ values were more variable among individuals of *Dickdellia* and ranged between -24.7‰ and -25.8‰. The reason for this is unclear, but it might be related to traces of carbonate (shell fragments, radula). Carbonate $\delta^{13}C$ values of an organism's shell are typically much higher than those of its tissue. This could explain why the snail $\delta^{13}C$ values were slightly higher than the values of the potential host (-26.0‰ and -27.2‰), which could also be explained by a small trophic carbon isotope enrichment (Peterson & Fry 1987).

The measured $\delta^{15}N$ values thus fit with a life history of *Dickdellia* as a *Colossendeis* parasite and not with a life history of a phoretic snail. However, our preliminary data are no more than a hint, because we used only a single *Dickdellia* egg cluster on a single *Colossendeis*, and this *per se* cannot lead to highly significant results. On the other hand, it is noteworthy that all of these data come from only one *Dickdellia–Colossendeis* sample and thus depict a real example of such an association.

Our results point to the necessity of checking for differences in the isotopic signatures of tissues potentially consumed by ectoparasites and of determining trophic fractionation values for individual taxonomic groups in future studies.

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Author contributions

RRM, TL and CM designed and directed the project. CM performed the laboratory work. RRM, TL and CM analysed the data and wrote the text.

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