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Molybdenum isotope signature of microbial nitrogen utilization in siboglinid tubeworms

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ABSTRACT

Many chemosynthesis-based communities prospering in deep-sea environments rely on the metabolic activity of sulfide-oxidizing bacteria. This is the case for vestimentiferan siboglinid tubeworms, whose demand for nutrition is satisfied predominantly by their endosymbiotic bacteria harbored in a specialized organ called the trophosome. Such chemosymbiosis leads to a significantly lower nitrogen isotope composition of the trophosome than in other types of soft tissue. However, the specific process of nitrogen utilization by siboglinids remains unclear. As a key element in the relevant enzymes (nitrogenase and nitrate reductase), molybdenum (Mo) is indispensable in the biogeochemical cycling of nitrogen. The Mo isotope composition ($\delta^{98}\text{Mo}$) of siboglinids is thus a potential proxy for decoding the processes involved in nitrogen metabolism. In this study, we found $\delta^{98}\text{Mo}$ values along the chitinous tube of the vestimentiferan siboglinid *Paraescarpia echinospica* from the Haima seeps of the South China Sea as negative as -4.59‰ ($-1.13\text{‰} \pm 1.75\text{‰}$, 1SD, $n = 19$)—the lowest $\delta^{98}\text{Mo}$ value ever reported for any kind of natural material. It is suggested that this extremely negative Mo isotope composition is caused by preferential utilization of isotopically light Mo by the tubeworm's endosymbionts or epibionts during nitrate reduction. Such Mo isotope signature could provide a means for identifying siboglinid tubeworms, a group of annelids that has previously escaped unambiguous identification due to the lack of mineralized skeleton, in the rock record.

INTRODUCTION

The dark depths of the ocean were once regarded as largely lifeless areas until the discovery of chemosynthesis-based deep-sea ecosystems at hydrothermal vents and methane seeps (Corliss et al., 1979; Paull et al., 1984).

In these hidden ecosystems, chemotrophic symbiotic bacteria provide nutrition for their metazoan hosts, taking advantage of reduced chemical compounds that sustain these deep-sea oases (Levin et al., 2016). In the past half-century, the research on these ecosystems has resulted in significant achievements in marine geology and biology, which have greatly expanded our understanding of life's evolution (Hryniewicz, 2022).

The dominant fauna at many hydrothermal vents and methane seeps are vestimentiferan

tubeworms, members of the annelid family Siboglinidae (Hilário et al., 2011). Adult siboglinids lack a functional digestive system, all nutrition being provided instead by their thiotrophic symbionts (sulfide-oxidizing bacteria) harbored within a specialized organ referred to as the trophosome. Like all metazoans, siboglinids require nitrogen, obtained through either nitrogen fixation or ammonium assimilation. Nitrogen fixation is common in marine cyanobacteria (Zerkle et al., 2011), while ammonium assimilation requires organisms to take up ammonium from the environment (Morse and Morin, 2005). It has been established that the host-symbiont interaction alters the nitrogen isotope composition of the trophosome (for *Paraescarpia echinospica*, $\Delta^{15}\text{N}_{\text{max}}$ [maximum difference of $\delta^{15}\text{N}$ between trophosome and other soft tissues] = 2.76‰ ; cf. Wang et al., 2022), a pattern that cannot be attributed to nitrogen uptake by the host (Saino and Ohta, 1989). It is also difficult for siboglinids to obtain ammonium or amino acids from the environment because they lack a digestive system, and the concentration of free amino acids in seawater is typically low (Amend and Shock, 1998).

One of the characteristics of the blood and coelomic fluids of siboglinids is the abundance of nitrate and nitrite, representing concentrations as much as two orders of magnitude higher than in ambient seawater (Pospesil et al.,

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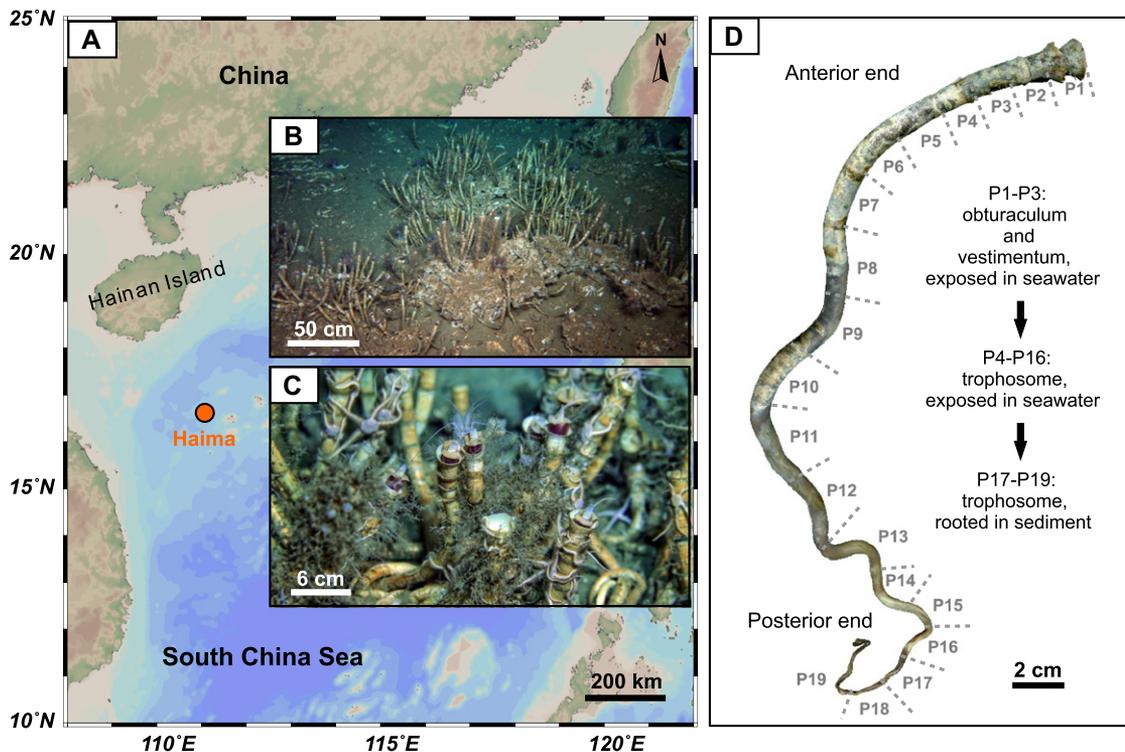


Figure 1. (A) Map of South China Sea with approximate sampling location (Haima seeps). Base map was generated by Ocean Data View (<https://odv.awi.de/>). (B,C) Aggregations of tubeworm *Paraescarpia echinospica* (modified from Wang et al., 2022). (D) Chitinous tube of *P. echinospica* investigated in this study. P1 to P19 represent subsections analyzed for trace elements and Mo isotopes. Soft-tissue sections and living environment corresponding to chitinous tube based on previous studies (Southward et al., 2002).

1998). Interestingly, nitrate can be reduced to nitrite and subsequently ammonia involving the enzyme nitrate reductase (Girguis et al., 2000). Nitrate reduction has been suggested to be the dominant pathway of nitrogen acquisition by siboglinids (Minic and Hervé, 2004).

Because nitrate reductase is a molybdenum (Mo)-containing enzyme in the biological nitrogen cycle (Ceccaldi et al., 2015), fractionation of Mo isotopes is likely to take place during microbial nitrate reduction in siboglinids. It has been suggested that microorganisms preferentially incorporate light Mo isotopes (Kendall et al., 2017), but to date, only one study exists that describes the utilization of nitrate and nitrogen fixation by cyanobacteria—the observed variability of Mo isotope ratios in cyanobacteria was found to range from $\sim 1.05\text{‰}$ to 2.10‰ ($\Delta^{98}\text{Mo}_{\text{cells-media}}$ [difference of $\delta^{98}\text{Mo}$ between cultured cells of the freshwater cyanobacterium *Anabaena variabilis* and culture medium] = -0.2‰ to -1.0‰ ; Zerkle et al., 2011).

Here, we investigate trace element distribution patterns and Mo isotope compositions along the chitinous tube of *P. echinospica* from the Haima seeps, South China Sea (Fig. 1; Tables S1 and S2 in the Supplemental Material¹). Previous studies have suggested the elemental distribution along the tube of vestimentiferan sibogli-

nids records local biogeochemical and microbial processes (Duperron et al., 2014; Bayon et al., 2020) associated with changing redox conditions at the interface between oxic bottom waters (at the organism's anterior end, which hosts the plume and the vestimentum) and anoxic pore water (at its posterior extension, where *P. echinospica* acquires dissolved sulfide and releases sulfate and hydrogen ions; Dattagupta et al. 2006). This general conceptual model presumes a link between biogeochemical processes within the soft tissue of tubeworms and the geochemical composition of the surrounding chitinous tube. However, little is known about the mechanisms that control trace element and isotope distribution patterns along the tube and how they relate to functional symbiotic microbial activity. In this study, we provide a new prospect on the applicability of Mo isotopes for decoding the processes involved in nitrogen utilization by seep siboglinids. Our new findings pave the way for the establishment of a novel geochemical proxy to recognize chemosymbiosis in the rock record.

RESULTS

Trace element contents of the studied chitinous tube of *P. echinospica* are shown in Figure 2 and Table S3. Almost all trace element contents increase toward both the anterior (oxic) and the posterior (anoxic) ends of the tube, probably reflecting the occurrence of strong redox gradients at the sediment-seawater interface where tubeworms live (cf. Duperron et al., 2014; Bayon et al., 2020). For the discussion below, we have subdivided the tube into four distinct sections

according to the portion of the tubeworm body that it encloses (P1–P3, corresponding to obturatorium and vestimentum, exposed to seawater; P4–P7 and P8–P16, trophosome, internal structure, anterior end; and P17–P19, trophosome, internal structure, posterior end; see Fig. 1 for labeled sections, and the Supplemental Material for the sampling strategy) based on physiological traits of *P. echinospica* (Southward et al., 2002; Bright and Lallier, 2010). At the environmental redox fronts (P1–P3 and P17–P19; Fig. 1), the total content of rare earth elements (REEs) is one order of magnitude higher than for P4–P16 (Fig. S1 in the Supplemental Material [see footnote 1]).

The $\delta^{98}\text{Mo}$ values decrease downward from the anterior end, reaching a minimum value of -4.59‰ at P4, then rise with some scatter toward the posterior end (Fig. 3; Table S4). Similar $\delta^{98}\text{Mo}$ values are observed from P17 to P19 ($\delta^{98}\text{Mo}$ values of P17, P18, and P19 are 1.29‰ , 1.50‰ , and 1.40‰ , respectively), which is close to the $\delta^{98}\text{Mo}$ values of surface sediment (1.17‰) and pore water (1.75‰ and 0.98‰) collected in the tubeworm habitat. Negative $\delta^{98}\text{Mo}$ excursions are observed for P9, P13, and P15.

DISCUSSION

Trace Element Composition of the Chitinous Tube Reveals Changing Redox Conditions

Elemental profiles along the chitinous tube of *P. echinospica* from the Haima seeps are similar to those for *Escarpiia southwardae* at the Regab pockmark in the Gulf of Guinea (Fig. 2; Bayon et al., 2020). The similarity between

¹Supplemental Material. Materials and methods, Tables S1–S5, Figure S1, and references. Please visit <https://doi.org/10.1130/22814594> to access the supplemental material, and contact editing@geosociety.org with any questions.

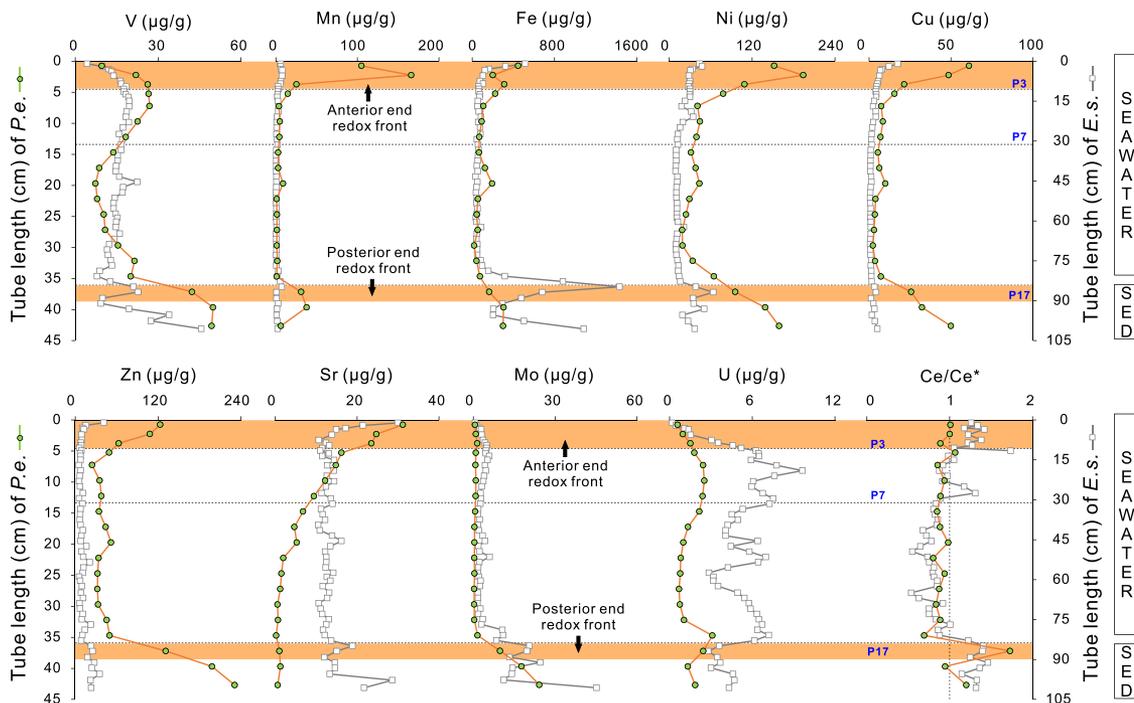


Figure 2. Trace element profiles along chitinous tube of *Paraescarpia echinospica* (P.e.; green circles); results of *Escarpia southwardae* (E.s.) are provided for comparison (white squares; Bayon et al., 2020). $Ce/Ce^* = 3 \times Ce_N / (2 \times La_N + Pr_N)$, where N refers to normalization of concentration against the standard Post-Archaean Australian Shale (PAAS). SED—sediment.

observed elemental patterns along the tube of different species from distinct locations is best interpreted as reflecting the presence of redox fronts at both the anterior and posterior ends of the chitinous tube (Duperron et al., 2014; Bayon et al., 2020). These redox interfaces are intimately connected to ambient microenvironmental conditions at seeps and physiological traits of siboglinids (Hilário et al., 2011). For instance, observed trace element enrichments at the anterior end, especially for redox-sensitive elements like vanadium (V) and Mo, could possibly relate to the presence of manganese (Mn) oxides, as inferred from high Mn contents (Crusius et al., 1996; Bayon et al., 2020). Additionally, enrichments in copper (Cu), nickel (Ni), and zinc (Zn) can be explained by local aerobic oxidation of methane at the anterior end of the tubeworm (Bayon et al., 2020). The posterior end of *P. echinospica* also displays significant enrichment of iron (Fe), consistent with increasing sulfide contents in the pore water at shallow sediment depth within the Haima seeps (Liu et al., 2020) or simply reflect higher trace element levels in pore waters relative to seawater. The content profile of uranium (U) is different from that of other trace elements due to its behavior in reducing sediments with its sensitivity to suboxic conditions (e.g., Algeo and Tribouillard, 2009). The decrease of strontium (Sr) contents between P1 and P11 could reflect the presence of minute crystals of authigenic aragonite precipitated on the chitinous tube as previously observed at other methane seeps (Haas et al., 2009). The observed REE patterns are consistent with the characteristics of other trace elements, reflecting both the relative contribution of oxic bottom waters and anoxic pore waters and the prefer-

ential utilization of light REEs in the anterior region of the tubeworm due to aerobic oxidation of methane (Wang et al., 2020; see Fig. S1).

Influence of Nitrate Reduction on Mo Isotope Distribution Patterns along the Tube

The $\delta^{98}\text{Mo}$ values along the chitinous tube of *P. echinospica* (-4.59‰ to 1.50‰ ; Fig. 3) are highly variable and different from those of a serpulid tube from Abu Dhabi, Persian Gulf (0.9‰ ;

Voegelin et al., 2009). In this study, the most negative $\delta^{98}\text{Mo}$ values obtained for the single specimen of chitinous tube of Haima siboglinid are the lowest ever reported values from Earth's surface environments (Fig. 4). The lowest previously reported $\delta^{98}\text{Mo}$ values as low as -4‰ stem from early Mesoproterozoic carbonates (Luo et al., 2021) and were attributed to preferential incorporation of isotopically light Mo during extensive precipitation of manganese and

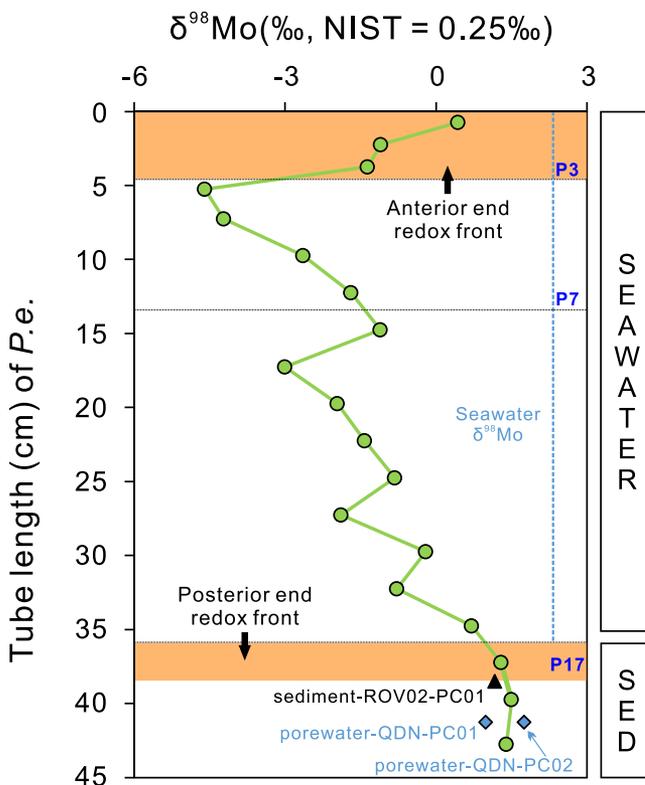


Figure 3. Molybdenum isotope compositions along chitinous tube of *Paraescarpia echinospica* (P.e.; green circles), average seawater (blue dotted line; Kendall et al., 2017), as well as pore water (blue diamonds) and sediment (black triangle) from study area. NIST—National Institute of Standards and Technology; SED—sediment.

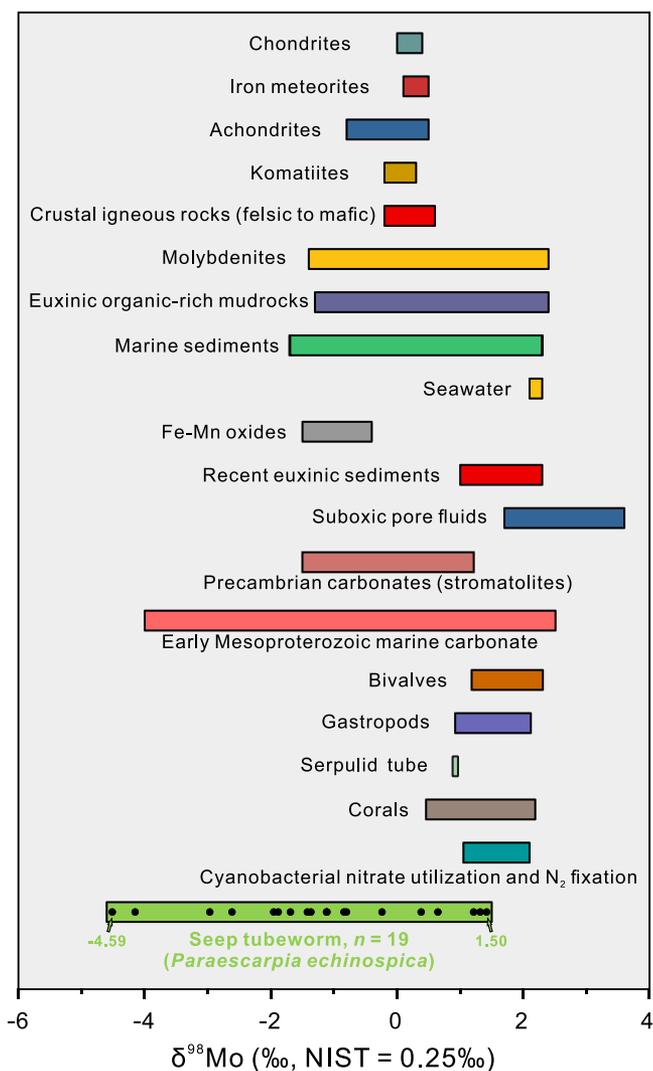


Figure 4. Molybdenum isotope compositions of various natural materials. See Table S5 (see text footnote 1) for sources of data. NIST—National Institute of Standards and Technology.

isotope composition in this section of the chitinous tube. An alternative explanation accounting for the observed Mo isotope variability and extremely low $\delta^{98}\text{Mo}$ values along the chitinous tube involves microbial nitrogen cycling at the outer tube wall of siboglinids. Indeed, growing evidence exists that the outer tube surface of siboglinids hosts abundant and highly diverse microbial communities of epibionts (Medina-Silva et al., 2018; Rincón-Tomás et al., 2020), which are part of the tubeworm's holobiont and could be involved in extensive nitrate reduction (Furst et al., 2021). While further work obviously is needed to better understand the microbial pathways controlling the acquisition of highly negative $\delta^{98}\text{Mo}$ signatures of siboglinid tubes, our findings provide promising evidence that Mo isotopes can be used to trace nitrogen cycling in chemosynthesis-based metazoans including their fossilized hard parts.

CONCLUSIONS AND PERSPECTIVES

Trace element and Mo isotope analyses of the chitinous tube of the siboglinid tubeworm *Paraescarpia echinospica* recovered at a methane seep provide information on both local microenvironmental conditions and the microbial activity of the siboglinid's endosymbionts or epibionts. For the first time, a relationship is established between microbial nitrogen cycling associated with siboglinids and Mo isotope fractionation, suggesting that nitrate reduction by either endosymbiotic sulfide-oxidizing bacteria in the trophosome and/or epibionts on the external tube wall can result in highly negative $\delta^{98}\text{Mo}$ values archived in the chitinous tube. Meanwhile, the findings presented here imply that the global Mo cycle can be strongly affected by local effects of nitrogen cycling, an observation warranting more comprehensive investigation in the future (cf. Howarth and Cole, 1985).

The close interdependence of siboglinids and their endosymbionts has been fascinating scientists for decades, yet it is difficult to constrain the first emergence of this type of chemosymbiosis based on morphological, molecular, ecological, and fossil evidence (Hilário et al., 2011). The emergence of tubeworms resembling siboglinids has been traced back to the Silurian for hydrothermal vents (Little et al., 1997) and to the Devonian for hydrocarbon seeps (Peckmann et al., 2005). However, molecular clock estimates shed doubt on the taxonomic affiliation of the Paleozoic tubular fossils with modern vestimentiferan siboglinids (Georgieva et al., 2019). In light of our findings, the application of the new Mo isotope proxy for microbial nitrogen utilization—potentially archived in carbonate-replaced former chitinous tubes—could help unravel the evolution of chemosymbiosis in tube-dwelling worms at hydrocarbon seeps.

iron (oxyhydr)oxides and subsequent diagenetic recycling. Interestingly, at the Haima seeps, the Mo isotope values of the anterior and posterior ends of the chitinous tube, also associated with increased Fe and Mn contents, are much higher than values of the middle part of the tube (Figs. 2 and 3). Such small-scale variability in the Mo isotope composition is unlikely to be explained solely by the presence of strong redox gradients. Instead, we suggest that the observed $\delta^{98}\text{Mo}$ variability along the chitinous tube and more specifically the excursion toward highly negative values in P4–P5 are best explained by a local physiological control (Table S5).

Molybdenum is a key element of nitrogenase and nitrate reductase and thus plays a crucial role in nitrogen cycling in marine environments. At the Haima seeps, the symbiotic thiotrophs associated with *P. echinospica* are not capable of nitrogen fixation but can perform dissimilatory nitrate reduction, denitrification, and ammonium assimilation (Li et al., 2021). Previous work on the nitrate assimilation pathway in siboglinids and associated excretion of nitrite, ammonia, and amino acids has suggested that ammonia

probably represents the main source of nitrogen for biosynthesis in the host and its symbionts (Girguis et al., 2000; Minic and Hervé, 2004). On this basis, we argue that the shift toward strong ^{98}Mo depletion in the tube of *P. echinospica* is best explained by nitrogen cycling related to nitrate and/or nitrite reduction by the thiotrophic endosymbionts, reflecting their strong dependency on high Mo uptake.

This finding suggests a close relationship between the physiology of siboglinids, the activity of endosymbionts, and nitrogen cycling. Although increasing toward the posterior end of the tube, $\delta^{98}\text{Mo}$ values between P4 and P16, corresponding to the whole trophosome, remain relatively low ($< \sim 0.7\text{‰}$; seawater $\delta^{98}\text{Mo}$: 2.3‰; Fig. 3), suggesting that nitrate reduction and associated Mo isotope fractionation could take place along the whole trophosome. In the lowermost parts the tube at its posterior end (P17–P19), $\delta^{98}\text{Mo}$ values are similar to the values of ambient surroundings (sediment: 1.17‰; pore water: 0.98‰ and 1.75‰), possibly indicating that local environmental conditions prevail over microbial nitrate reduction in controlling the Mo

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