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## Comparative bioaccumulation of trace elements between *Nautilus pompilius* and *N. macromphalus* (Cephalopoda: Nautiloidea) from Vanuatu and New-Caledonia

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1 **Comparative bioaccumulation of trace elements between *Nautilus pompilius* and *N.***  
2 ***macromphalus* (Cephalopoda: Nautiloidea) from Vanuatu and New-Caledonia**

3

4 Running title: influence of the environment on bioaccumulation of trace elements in *Nautilus*

5

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26

27 **ABSTRACT:** The concentrations of sixteen trace elements were investigated and compared  
28 for the first time in the digestive and excreting tissues of two *Nautilus* species (Cephalopoda:  
29 Nautiloidea) from two geologically contrasted areas: 1) *N. macromphalus* from New  
30 Caledonia, a region characterised by its richness in nickel ores and its lack of tectonic  
31 activities and 2) *N. pompilius* from the Vanuatu archipelago showing high volcanic and  
32 tectonic activities. In both *Nautilus* species, results clearly highlighted that the digestive gland  
33 played a key role in the bioaccumulation and storage of Ag, Cd, Ce, Co, Cu, Fe, La, Nd, V  
34 and Zn whereas As, Cr, Mn, Ni, Pb and Se were accumulated in a greater extent in the  
35 excreting tissues (i.e. pericardial and renal appendages). Despite contrasting environments, no  
36 significant difference ( $P < 0.05$ ) was found between the two *Nautilus* species in the  
37 concentrations of most of the essential and non essential elements, including Ni and  
38 associated metals in Ni ores (i.e. Co and Mn). As nautilus lives on the outer shelf of barrier  
39 reefs, these results strongly support the hypothesis that the New Caledonian lagoon traps the  
40 major amount of the trace elements derived from natural erosion and the intense mining  
41 activities conducted on land. In contrast, the concentrations of the rare earth elements (Ce, La,  
42 and Nd) were significantly higher in *N. pompilius* than in *N. macromphalus*, probably as a  
43 result of the local enrichment of Vanuatu waters by specific environmental processes, such as  
44 volcanism or upwelling.

45

46 **Keywords:** metals, metalloids, cephalopods, detoxification, excretion, South Pacific

## 47 1. INTRODUCTION

48

49 Marine organisms are exposed to trace elements that are present in their diet and dissolved in  
50 seawater. This double exposure results in element accumulation in their different tissues  
51 whether or not these elements are essential to the metabolism (Rainbow, 2002). This process,  
52 widely described as bioaccumulation can vary greatly according to the specificities of the  
53 organisms such as age/size, sex and lifestyle, and according to the relative bioavailability of  
54 the metal in diet and seawater (Rainbow and Wang, 2001). Comparative analysis of trace  
55 element concentrations between closely related species living in different geographical areas  
56 can therefore allow at assessing the influence of the environment on their bioaccumulation.

57 Among the marine species, cephalopods belong to a molluscan group comprising ca. seven  
58 hundred species (Boyle and Rodhouse, 2005), in which high concentrations of trace elements  
59 such as Ag, Cd, Cu or Zn have generally been recorded (Martin and Flegal, 1975; Finger and  
60 Smith, 1987; Miramand and Bentley, 1992; Yamada et al., 1997; Bustamante et al., 1998;  
61 Dorneles et al., 2007). Indeed, several studies have demonstrated that the digestive and the  
62 excretory organs of cephalopods play a major role in the bioaccumulation of trace elements,  
63 as these organs are deeply involved in the assimilation processes, detoxification and storage  
64 of both essential and non-essential metals (Miramand and Guary, 1980; Miramand and  
65 Bentley, 1992; Bustamante et al., 2000, 2002a,b, 2004).

66 In the present work, we focused on the bioaccumulation of trace elements in the *Nautilus*  
67 genus. Nautiluses are scavenger cephalopods, inhabiting the outer shelf of barrier reefs in the  
68 Indo-Pacific area, generally at a depth of 300 to 500 meters (O'Dor et al., 1993; Norman,  
69 2000). In spite of its evolutionary interest (*Nautilus* is the last representative of ectocochleate  
70 cephalopods, i.e. considered as a “living fossil”; Boyle and Rodhouse, 2005) and its  
71 physiological specificities (slow digestion and most of the excretory processes taking place in

72 the pericardial appendages; Schipp et al., 1985; Westermann et al., 2002), only very few  
73 studies deal with the bioaccumulation of trace elements in this ancestral genus. High  
74 concentrations of several metals and metalloids were reported in the digestive and excretory  
75 tissues of *Nautilus macromphalus* (Bustamante et al., 2000), a species endemic to the New  
76 Caledonian waters. These authors suggested that the high metal concentrations may be due to  
77 a high exposure in the metal-rich conditions in the surrounding New Caledonian waters.  
78 Indeed, the largest resources of Ni as laterites in the world are present in New Caledonia  
79 where open-cast mining is conducted since the 20<sup>th</sup> century, leading to a dramatic increase of  
80 metal concentrations in coastal waters and, subsequently, the metal burdens in marine biota  
81 (Labrosse et al., 2000; Metian et al., 2008). However, it is not clear whether metal  
82 accumulation observed in *N. macromphalus* reflected the impact of metal contamination  
83 through human activities on the outer shelf waters of New Caledonia or either if it is more due  
84 to a natural progressive bioaccumulation during nautilus long life span (*viz.* 10-15 years).

85  
86 The aim of the present study was therefore to determine the bioaccumulation of a large range  
87 of elements in the genus *Nautilus* comparing two species from geologically contrasted areas:  
88 1) *N. macromphalus* from New Caledonia which is characterised by its richness in nickel ores  
89 and its lack of tectonic activities and 2) *N. pompilius* from the Vanuatu archipelago which  
90 shows high volcanic and tectonic activities (Harrison et al, 1996). The analysed elements  
91 comprised 11 metals (Ag, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn), 2 metalloids (As and  
92 Se) and 3 rare earth elements (Ce, La and Nd). These elements were selected for comparison  
93 with other species and other published works concerning Nautilus (e.g. Miramand et al. 1992,  
94 Bustamante et al. 2000, Ichihashi et al. 2001). The concentrations and relative contents of  
95 these different trace elements were determined in the digestive and the excretory tissues (i.e.  
96 pericardial and renal appendages) and compared between both nautilus species. The

97 specificities and the potential influence of the environment on bioaccumulation of these trace  
98 elements in the *Nautilus* genus are discussed.

99

## 100 **2. MATERIALS AND METHODS**

### 101 **a) Biological material**

102 Three *Nautilus pompilius* and five *Nautilus macromphalus* specimens were collected  
103 respectively off Santo island coast (Vanuatu, 15° 40' 522 S, 167° 02' 838 E, depth: 334m) and  
104 on the outer shelf of New-Caledonia (22° 20' 500 S, 166° 13' 400 E, depth: 300m) in May  
105 2006 (Figure 1). Each individual was measured and weighted prior to dissection. The main  
106 features of the eight specimens sampled are reported in Table 1.

107 All specimens were dissected aseptically and for each specimen one pericardial appendage,  
108 one renal appendage and a fraction of the digestive gland were immediately frozen in liquid  
109 nitrogen and stored at -80°C for metal analyses.

110

### 111 **b) Analytical procedure**

112 Tissue samples were freeze-dried. Aliquots of the samples ranging from 20 to 300 mg were  
113 digested using a 3:1 v:v nitric-hydrochloric acid mixture with 65% ultrapure HNO<sub>3</sub> and  
114 ultrapure 70% HCl. The acidic digestion was performed overnight under ambient temperature  
115 and then heated in a microwave during 30 minutes, with increasing temperature until 105°C,  
116 and 15 minutes at 105°C (1200 Watts). After the mineralization process, each sample was  
117 diluted to 30 ml or 50 ml with milli-Q quality water, according to the volume of acid added to  
118 the mineralization (3 ml and 4.5 ml. respectively).

119 Ag, As, Cd, Ce, Co, Cr, Cu, Fe, La, Mn, Nd, Ni, Pb, Se, V and Zn were analysed either by  
120 ICP-OES (Varian<sup>®</sup> Vista-Pro) and ICP-MS (Varian<sup>®</sup> Ultra Mass 700). Reference tissues,  
121 dogfish liver DOLT-3 (NRCC), lobster hepatopancreas TORT-2 (NRCC) and Bush Branches

122 and Leaves (GBW 07602) were treated and analysed in the same way as the samples. Results  
123 were in good agreement with the certified values, and the standard deviations were low,  
124 proving good repeatability of the method. The results for standard reference materials  
125 displayed recoveries of the elements ranging from 81% to 119% (n=10). For each set of  
126 analyses, blanks were included in each analytical batch. The detection limits ( $\mu\text{g}\cdot\text{g}^{-1}$  dry wt)  
127 for ICP-OES were 8.3 (As, Fe, Zn), 3.3 (Ag, Se), 1.67 (Pb, V), 0.83 (Cd, Co, Cr, Cu, Mn, Ni),  
128 and for ICP-MS, they were 0.150 (Ni, V), 0.065 (Cd, Co, Cr, Cu, Mn, Pb), 0.033 (Ag), 0.017  
129 (Nd), 0.008 (Ce, La). Average element concentrations are presented with standard deviations.  
130 All trace element concentrations are given on a dry weight basis ( $\mu\text{g}\cdot\text{g}^{-1}$  dry wt).

131

### 132 *Data analyses*

133 Statistical analyses were performed using MINITAB 13.2 Software. Comparison of trace  
134 element concentrations in nautilus tissues between sites was tested by 1-way ANOVA.  
135 Hypothesis of normal distribution was tested using the Anderson-Darling test and equality of  
136 variance by the Bartlett test. Pearson coefficients were calculated between trace elements in  
137 each tissue. The significance level for statistical analyses was always set at  $\alpha = 0.05$ .

138

## 139 **3. RESULTS**

140

### 141 *Trace element concentrations in the tissues*

142 Metal concentrations in the digestive gland, the renal appendages and the pericardial  
143 appendages of *N. macromphalus* and *N. pompilius* are reported in Table 2. La, Ce, and Nd  
144 concentrations fell below the detection limit in the renal appendages. Among the three tissues  
145 analysed, the digestive gland was the major site of accumulation for Ag, Cd, Ce, Co, Cu, Fe,  
146 La, Nd, V and Zn (Table 2). The digestive gland also concentrated As and Ni at

147 concentrations close to the highest ones recorded in the other tissues. As, Cr, Mn, Ni, Pb, and  
148 Se concentrations were remarkable in excreting tissues. More specifically, the renal  
149 appendages exhibited the highest Mn and Se concentrations, whereas As, Ni, and Pb were  
150 concentrated at similar concentrations within both renal and pericardial appendages. Finally,  
151 the pericardial appendages were the major site of accumulation for Cr.

152

### 153 *Comparison between species*

154 Generally, the pattern of trace element concentrations was the same in both *N. macromphalus*  
155 and *N. pompilius* tissues. However, there were few exceptions and more particularly in the  
156 digestive gland: firstly, Ce, Fe, La, and Nd concentrations were significantly ( $P < 0.05$ ) higher  
157 in *N. pompilius*; secondly, Cd and Cr concentrations were significantly ( $P < 0.05$ ) higher in *N.*  
158 *macromphalus* (Table 3).

159 In the case of the pericardial appendages, As, La, and Nd concentrations were significantly  
160 higher ( $P < 0.05$ ) in *N. pompilius* (Table 3). For the renal appendages, Cu was the only element  
161 for which a significant difference was detected, *N. pompilius* having significantly higher  
162 concentrations than *N. macromphalus* (Table 3).

163

### 164 *Relative proportion of trace elements between tissues*

165 Among the three tissues considered, the digestive gland contained the largest proportions of  
166 most of the trace elements with 73 to 81% of Ag, 90 to 91% of Cd, 61 to 79% of Ce, 80 to  
167 86% of Co, 67 to 72% of Cu, 90 to 93% of Fe, 72 to 81% of La, 55 to 75% of Nd, 56 to 67 of  
168 V, 81% to 82% of Zn in both *Nautilus* species (Fig. 2). The only exception were As, Cr, Mn,  
169 Ni, Se, and Pb which were mainly contained in the excreting tissues, i.e. renal and pericardial  
170 appendages (Fig. 2). The renal appendages were the major site of accumulation of Mn, Ni,  
171 and Se (i.e. 74 to 89% for Mn, 49 to 54% for Ni; 61 to 73% for Se) while the pericardial



172 appendages accumulated the highest relative proportion of Cr (i.e. 63 to 74%). Although the  
173 concentrations of some of the trace elements were high in the excretory organs, these tissues  
174 contained in fact low amounts of metals because of their small masses.

175

#### 176 *Correlations between metals*

177 Table 4 shows the correlation between trace elements within the three tissues. It is noteworthy  
178 that the rare earth elements were correlated between each other within the digestive gland ( $r >$   
179  $0.985$  and  $P < 0.001$  in all cases) and the pericardial appendages ( $r > 0.922$  and  $P < 0.003$  in all  
180 cases). In the digestive gland, rare earth elements varied positively with Fe and Mn ( $r > 0.918$   
181 and  $r > 0.807$  and  $P < 0.004$  and  $P < 0.028$ , respectively) but negatively with As ( $r > -0.726$  and  
182  $P = 0.047$ ). Cu correlated positively with Zn ( $r = 0.834$  and  $P = 0.020$  in all cases) but negatively  
183 with Se in this tissue ( $r = -0.809$  and  $P = 0.028$  in all cases).

184 In the renal appendages, correlations were weak, Mn varied positively with Cd ( $r = 0.833$ ,  
185  $P = 0.010$ ) and Zn ( $r = 0.735$ ,  $P = 0.038$ ), Fe with Zn ( $r = 0.833$ ,  $P = 0.010$ ) and Pb with Cr  
186 ( $r = 0.936$ ,  $P = 0.001$ ) but negatively with V ( $r = -0.753$ ,  $P = 0.031$ ; Table 3).

187 In the pericardial appendages, Cd concentrations correlated positively with Cr ( $r = 0.897$ ,  
188  $P = 0.006$ ), Ni ( $r = 0.965$ ,  $P < 0.001$ ), and V ( $r = 0.931$ ,  $P = 0.002$ ).

189

#### 190 **4. DISCUSSION**

191

192 The present extensive analysis of sixteen trace element concentrations in *N. macromphalus*  
193 and *N. pompilius* digestive and excreting tissues clearly highlighted that these organs  
194 constitute important tissues for the bioaccumulation of rare earth elements, metals and  
195 metalloids. Such results support the hypothesis of efficient regulation processes for non

196 essential metals in Nautilidae (Bustamante et al., 2000) even if, among the eight studied  
197 specimens, the variability of metal concentrations was relatively high (Table 2).

198 In cephalopods, the digestive system plays a major role in both the digestive and the  
199 detoxification processes: on the one hand, the digestive gland synthesizes the digestive  
200 enzymes and is a place of absorption and assimilation of nutrients (Boucaud-Camou and  
201 Boucher-Rodoni, 1983; Boucher-Rodoni and Boucaud-Camou, 1987); on the other hand, this  
202 organ is involved in the storage and detoxification of trace elements (Bustamante et al.,  
203 2002ab, 2004). Our investigations on trace element concentrations also confirmed the key role  
204 of the digestive gland of both *N. macromphalus* and *N. pompilius* in the metabolism of most  
205 of the analysed trace elements (i.e. Ag, Cd, Ce, Co, Cu, Fe, La, Nd, V and Zn), as it is also the  
206 case in more recent cephalopods, i.e. belonging to the Coleoidae (e.g. Ghiretti-Magaldi et al.,  
207 1958; Martin and Flegal, 1975; Miramand and Guary, 1980; Smith et al., 1984; Finger and  
208 Smith, 1987; Miramand and Bentley, 1992; Seixas et al., 2005a; Pierce et al., 2008).

209 Our results also stressed the potential of excreting tissues (i.e. renal and pericardial  
210 appendages) to accumulate several elements such as As, Cr, Mn, Ni, Pb, and Se. In contrast to  
211 the Coleoidae, in nautilus the renal appendages are specialized in the storage of calcium and  
212 the pericardial appendages play a major role in the evacuation of the metabolic wastes  
213 (Schipf and Martin, 1987). These organs are known to harbour a dual bacterial symbiosis  
214 (Pernice et al., 2007) involving spirochaetes and betaproteobacteria. The exact mechanisms  
215 by which these bacterial symbionts are involved in nautilus excretion are still enigmatic.  
216 Nevertheless, it is widely admitted that micro-organisms can play an important role in the  
217 incorporation of trace elements (Worms et al., 2006). Accumulation of Co, Fe and Ni by  
218 betaproteobacterial symbionts of the pericardial appendages of *N. macromphalus* has already  
219 been observed by electronic microscopy (Pernice et al., in preparation). However, it remains  
220 unclear if this accumulative process is only passive (viz. through adsorption processes) or

221 active (viz. effective absorption of trace elements). Indeed, the betaproteobacterial symbionts  
222 present in nautilus tissues could play a role in the detoxification and storage of some metals  
223 such as Co, Fe and Ni (Table 2). Such processes could involve enzymatic reduction and/or  
224 complexation with organic compounds (Pernice et al., 2006), leading to the storage of these  
225 metals at high concentrations in the excreting tissues, independently of the nautilus species.  
226 The role of this bacterial symbiosis on trace element bioaccumulation and detoxification in  
227 nautilus excreting tissues clearly deserves further investigations.

228 Although Nautilidae have been previously reported for their special abilities to accumulate  
229 heavy metals at relatively elevated concentrations in their tissues (Bustamante et al., 2000),  
230 this study is the first comparative approach aiming to compare trace element concentrations in  
231 two *Nautilus* species from two geographically close areas (viz. distant from less than 1000  
232 kms). Both species potentially share common trophic features but, at the same time, they live  
233 in geologically different environments (richness in nickel ores and lack of tectonic activities  
234 in New Caledonia and high volcanic and tectonic activities in Vanuatu), potentially resulting  
235 in a different degree of “natural” contamination. New Caledonia is currently the third largest  
236 producer of Ni in the world (Dalvi et al., 2004) and mining activities associated to a strong  
237 natural erosion have resulted in the enrichment of Ni and associated metals in Ni ores (i.e. Co,  
238 Cr, Cu, Fe and Mn) and some other extracted metals (i.e. Ag and Pb) in the coastal waters and  
239 consequently in the lagoon food webs (Monniot et al., 1994; Fernandez et al., 2006; Hédouin  
240 et al., 2006; Metian et al., 2008). Conversely, it is likely that the seawater concentrations of  
241 most of these metals are very low in Santo islands waters and around Vanuatu in general  
242 according to the absence of industrial and mining activities in this area (Harrison et al., 1996).  
243 Surprisingly, in the present study, very little difference appeared in the sixteen trace element  
244 concentrations between *N. macromphalus* from New Caledonia and *N. pompilius* from  
245 Vanuatu. Interestingly, there was no significant difference between both *Nautilus* species for

246 Ag, Co, Mn, Ni, Pb, Se, V and Zn (Table 3) indicating that the geographical origin does not  
247 seem to be a major factor contributing to interspecific variations of these trace element  
248 concentrations. As nautilus lives on the outer shelf of barrier reefs and rarely penetrates into  
249 lagoons (Saunders and Ward, 1987; Norman, 2000), our results strongly supported the  
250 hypothesis that the New Caledonian lagoon traps the major amount of the trace elements  
251 derived from the island (Ambatsian et al., 1997). To the best of our knowledge, there are no  
252 published data in the open literature concerning trace elements in organisms from the oceanic  
253 waters off New Caledonia, except for two marine mammal species (Bustamante et al., 2003).  
254 These authors reported very low concentrations of Co, Cr and Ni in the marine mammal  
255 tissues which suggested that mining activity in New Caledonia does not seem to be a  
256 significant source of contamination in the oceanic domain. Overall, the global geologic  
257 contagion due to the mining activity in New Caledonia seems to be limited to the shoreline  
258 waters and to the inner lagoon (Fernandez et al., 2006).  
259 More interestingly, the concentrations of rare earth elements were significantly different  
260 between the digestive gland and the pericardial appendages of *N. macromphalus* and *N.*  
261 *pompilius*. F-transition elements of rare earths have similar physicochemical properties, and it  
262 is therefore not surprising that they correlate to each other (Table 4). Our results suggested  
263 that rare earth elements would reflect the characteristics of the surrounding environment.  
264 Very little is known about the concentrations of Ce, La and Nd in cephalopods, which varied  
265 from 0.045 to 1.95  $\mu\text{g}\cdot\text{g}^{-1}$  dwt in the digestive gland of the squid *Stenoteuthis oualaniensis*  
266 (Ichihashi et al., 2001). In fact, rare earth elements have chemistry and sorption/complexation  
267 characteristics analogous to transuranic elements such as americium or plutonium (Choppin  
268 Torres and Chopin, 1984; Kim et al., 1989; Buckau et al., 1992). In Coleoid cephalopods,  
269 transuranic elements also accumulate in the digestive and excreting tissues, in particular in the  
270 branchial heart and their appendages (Guary et al., 1981; Guary and Fowler, 1982;

271 Bustamante et al., 2006). It is therefore not surprising that they accumulate at relatively high  
272 concentrations in nautilus digestive gland and pericardial appendage.

273 Besides Ce, La and Nd, the concentrations of As, Cu and Fe were also higher in *N. pompilius*  
274 than in *N. macromphalus*. It is difficult to ascertain the reasons of such a difference but this  
275 might also be linked to geological features reflecting a potential enrichment of Vanuatu  
276 waters by local environmental processes, such as volcanism or upwelling (Malinovsky and  
277 Markevich, 2007). In fact, only two trace elements, Cd and Cr, displayed significantly higher  
278 concentrations in *N. macromphalus* than in *N. pompilius* tissues (Table 2). Therefore, the  
279 reasons of such interspecific differences in bioaccumulation, i.e. higher concentrations of  
280 trace elements in one species, remain unclear whereas an absence of difference could be  
281 explained by the similar physiology of these two species (Ward, 1988; Norman, 2000).

282 As for other marine organisms, in cephalopods, trace element concentrations may vary with  
283 biological and environmental factors such as temperature, salinity, season, age (size), sex,  
284 lifestyle and geographical origin (e.g. Monteiro et al., 1992; Bustamante et al., 1998;  
285 Raimundo et al., 2004; Seixas et al., 2005b; Pierce et al., 2008). Further investigations are  
286 needed to understand how exactly these factors affect their concentrations in the studied  
287 species. However, among these factors, individual size/age is known to be of primary  
288 importance in invertebrates (e.g. Warnau et al., 1995; Bustamante and Miramand, 2005;  
289 Hédouin et al., 2007). In the present study, the size as well as the two others main features  
290 measured (sex and weight) were globally homogenous among the eight studied specimens.  
291 Because nautilus are long lived animals, it would be striking to determine the influence of  
292 the size/age on trace elements bioaccumulation.

293 Sources of trace elements to nautilus can be (1) seawater, as it passes through the skin and  
294 through the gills during respiration, and (2) food, which is now considered as a major route  
295 for metal accumulation in marine animals, including both invertebrates and fish (Koyama et

296 al., 2000; Wang, 2002; Bustamante et al., 2002a, 2004). Knowledge of trace element  
297 speciation in the direct environment of nautilus is a key factor to ascertain the relative  
298 importance of each uptake pathways. Concerning the first pathway (i.e. seawater), further  
299 aquatic toxicity studies are needed to determine the fraction of total metal dissolved in  
300 seawater (the 'toxic fraction') that react with, and is transported across biological membranes  
301 such as nautilus gills. Concerning the second pathway (i.e. food), our comparative analysis  
302 suggested that the metal discharges due to intense mining activities conducted on land in New  
303 Caledonia are not easily transferred in the food web of nautilus. Therefore, to assess the  
304 impact of mining activities on cephalopods in the region of New Caledonia, further studies  
305 should be conducted on more coastal species known to be resident in the impacted areas, such  
306 as Sepiidae, Sepiolidae or Octopodidae spending all of their life in the waters of the New  
307 Caledonian lagoon.

308

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310

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318

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526 Table 1. Characteristics of the sampled specimens of *Nautilus pompilius* and *N.*  
 527 *macromphalus*.

528

<b>Species</b>	<b>Sex</b>	<b>Length (mm)</b>	<b>Weight (g)</b>
<i>N. pompilius</i>			
1	Male	177	825
2	Male	151	660
3	Male	171	796
<i>N. macromphalus</i>			
1	Male	164	766
2	Female	162	676
3	Male	173	870
4	Male	160	758
5	Male	140	480



529 Table 2: Trace elements concentrations (Mean  $\pm$  SE;  $\mu\text{g g}^{-1}$  dry wt) in the tissues of Nautiloidae from this study and from the literature. The  
530 highest values are in bold. dl: detection limit; NA: not analysed; # only one value.

531

Tissue	Rare earth elements			Non essential metals			Essential metals and metalloids									
	La	Ce	Nd	Ag	Cd	Pb	As	Co	Cr	Cu	Fe	Mn	Ni	Se	V	Zn
<i>Nautilus macromphalus</i> (Bustamante et al. 2000)																
Digestive gland	NA	NA	NA	4.45 $\pm$ 2.68	<b>45.1<math>\pm</math>13.2</b>	0.46 $\pm$ 0.17	166 $\pm$ 26	<b>7.8<math>\pm</math>3.6</b>	4.4 $\pm$ 1.1	<b>106<math>\pm</math>46</b>	<b>666<math>\pm</math>231</b>	10.1 $\pm$ 1.7	16.3 $\pm$ 7.8	NA	<b>8.8<math>\pm</math>2.0</b>	<b>672<math>\pm</math>208</b>
Pericardial appendages	NA	NA	NA	<b>20.3<math>\pm</math>6.2</b>	2.16 $\pm$ 1.63	1.00 $\pm$ 0.82	<b>260<math>\pm</math>111</b>	1.31 $\pm$ 0.80	<b>8.25<math>\pm</math>1.55</b>	27.2 $\pm$ 33.4	78 $\pm$ 46	25.1 $\pm$ 23.0	5.64 $\pm$ 3.54	NA	0.18 $\pm$ 0.09	22.4 $\pm$ 26.6
Renal appendages	NA	NA	NA	1.80 $\pm$ 2.22	1.87 $\pm$ 1.23	<b>1.06<math>\pm</math>0.88</b>	112 $\pm$ 65	0.43 $\pm$ 0.37	2.54 $\pm$ 2.88	7.1 $\pm$ 10.2	343 $\pm$ 160	<b>77.1<math>\pm</math>54.1</b>	<b>22.7<math>\pm</math>13.9</b>	NA	1.02 $\pm$ 0.72	11.0 $\pm$ 16.8
<i>N. macromphalus</i> (Present study)																
Digestive gland	<b>0.30<math>\pm</math>0.07</b>	<b>0.25<math>\pm</math>0.11</b>	<b>0.19<math>\pm</math>0.07</b>	<b>9.42<math>\pm</math>3.91</b>	<b>51.5<math>\pm</math>3.5</b>	0.98 $\pm$ 0.15	<b>257<math>\pm</math>19</b>	<b>4.69<math>\pm</math>1.51</b>	2.95 $\pm$ 0.35	<b>263<math>\pm</math>78</b>	<b>353<math>\pm</math>63</b>	10.9 $\pm$ 1.3	14.0 $\pm$ 1.04	2.65 $\pm$ 0.90	<b>14.8<math>\pm</math>2.28</b>	<b>388<math>\pm</math>61</b>
Pericardial appendages	0.11 $\pm$ 0.02	0.17 $\pm$ 0.05	0.15 $\pm$ 0.05	1.82 $\pm$ 0.54	1.93 $\pm$ 0.76	1.57 $\pm$ 0.11	169 $\pm$ 19	0.65 $\pm$ 0.09	<b>9.59<math>\pm</math>3.35</b>	73.7 $\pm$ 9.9	19 $\pm$ 1	12.7 $\pm$ 2.9	6.93 $\pm$ 1.37	3.72 #	5.62 $\pm$ 2.26	56.5 $\pm$ 1.8
Renal appendages	<dl	<dl	<dl	0.44 $\pm$ 0.12	3.39 $\pm$ 1.16	<b>2.11<math>\pm</math>0.51</b>	152 $\pm$ 17	0.18 $\pm$ 0.03	2.57 $\pm$ 0.36	27.6 $\pm$ 4.2	20 $\pm$ 3	<b>68.2<math>\pm</math>6.2</b>	<b>19.7<math>\pm</math>2.39</b>	<b>9.84<math>\pm</math>0.94</b>	1.54 $\pm$ 0.13	35.2 $\pm$ 2.8
<i>N. pompilius</i> (Present study)																
Digestive gland	<b>1.00<math>\pm</math>0.14</b>	<b>1.58<math>\pm</math>0.32</b>	<b>0.92<math>\pm</math>0.10</b>	<b>5.65<math>\pm</math>1.04</b>	<b>74.3<math>\pm</math>6.8</b>	0.62 $\pm$ 0.07	210 $\pm$ 16	<b>3.85<math>\pm</math>1.04</b>	1.37 $\pm$ 0.24	<b>311<math>\pm</math>61</b>	<b>625<math>\pm</math>51</b>	15.4 $\pm$ 1.7	9.77 $\pm$ 0.07	2.35 $\pm$ 0.84	<b>8.42<math>\pm</math>0.57</b>	<b>470<math>\pm</math>52</b>
Pericardial appendages	0.24 $\pm$ 0.03	0.42 $\pm$ 0.05	0.31 $\pm$ 0.01	1.75 $\pm$ 0.07	2.39 $\pm$ 0.37	<b>1.24<math>\pm</math>0.24</b>	<b>323<math>\pm</math>53</b>	0.79 $\pm$ 0.07	<b>9.30<math>\pm</math>3.87</b>	99.7 $\pm$ 2.4	21 $\pm$ 3	24.1 $\pm$ 4.9	7.02 $\pm$ 0.79	2.49 #	4.94 $\pm$ 0.74	58.2 $\pm$ 2.7
Renal appendages	<dl	<dl	<dl	0.35 $\pm$ 0.03	5.93 $\pm$ 0.31	0.99 $\pm$ 0.21	292 $\pm$ 80	0.18 $\pm$ 0.03	1.98 $\pm$ 0.08	53.3 $\pm$ 4.6	24 $\pm$ 3	<b>307<math>\pm</math>83</b>	<b>19.6<math>\pm</math>0.97</b>	<b>13.3<math>\pm</math>6.12</b>	1.82 $\pm$ 0.02	42.7 $\pm$ 1.5

532 Table 3. One-way ANOVA comparison of trace element concentrations in *Nautilus pompilius*

533 (Np) and in *N. macromphalus* (Nm). NS: not significant.

534

Element	Digestive gland	Pericardial appendages	Renal appendages
Rare earth elements			
Ce	Np > Nm (F= 19.58 ; p= 0.007)	NS	-
La	Np > Nm (F= 23.40; p= 0.005)	Np > Nm (F= 0.609; p= 0.025)	-
Nd	Np > Nm (F= 25.13 ; p= 0.004)	Np > Nm (F= 6.54 ; p= 0.051)	-
Essential elements			
As	NS	Np > Nm (F= 9.58 ; p= 0.027)	NS
Co	NS	NS	NS
Cr	Nm > Np (F= 11.34 ; p= 0.020)	NS	NS
Cu	NS	NS	Np > Nm (F= 15.58; p=0.008)
Fe	Np > Nm (F= 10.09 ; p= 0.025)	NS	NS
Mn	NS	NS	NS
Ni	NS	NS	NS
Se	NS	NS	NS
V	NS	NS	NS
Zn	NS	NS	NS
Non essential elements			
Ag	NS	NS	NS
Cd	Nm > Np (F= 0.740 ; p= 0.023)	NS	NS
Pb	NS	NS	NS

535 Table 4: Correlations between trace elements within the different tissues of nautilus.

536 Not underlined: p<0.05; underlined: p<0.001.

537

Elements	Digestive gland	Renal appendages	Pericardial appendages
La	+ <u>Ce</u> , + <u>Nd</u> , - As, + <u>Fe</u> , + <u>Mn</u>	<dl	+ <u>Ce</u> , + <u>Nd</u> , + <u>Mn</u>
Ce	+ <u>Nd</u> , - <u>As</u> , + <u>Fe</u> , + <u>Mn</u> ,	<dl	+ <u>Nd</u> , + <u>Co</u> , + <u>La</u>
Nd	+ <u>Fe</u> , + <u>Mn</u>	<dl	+ <u>Ce</u> , + <u>Co</u> , + <u>La</u>
Cu	+ Zn, -Se	-	-
Fe	+ Mn, -V, + <u>La</u> , + <u>Ce</u> , + <u>Nd</u>	+ Zn	+ Ag, + <u>Mn</u> , + Cr
Pb	+ V	- V, + <u>Cr</u>	-
Mn	+ La, + Ce, + Nd, + Fe	+ Cd, +Zn	+ La, + <u>Fe</u>
Cd	-	+ <u>Mn</u>	+ <u>Ni</u> , + <u>V</u> , + <u>Cr</u>

538

539 <dl: concentrations below the detection limit.

540 **Caption to figures**

541

542 Figure 1: Location of *Nautilus pompilius* and *N. macromphalus* specimens' collection.

543

544 Figure 2: Relative distribution of the trace elements between the digestive gland (DG),

545 pericardial appendages (PA) and renal appendages (RA) of *Nautilus pompilius* from Vanuatu

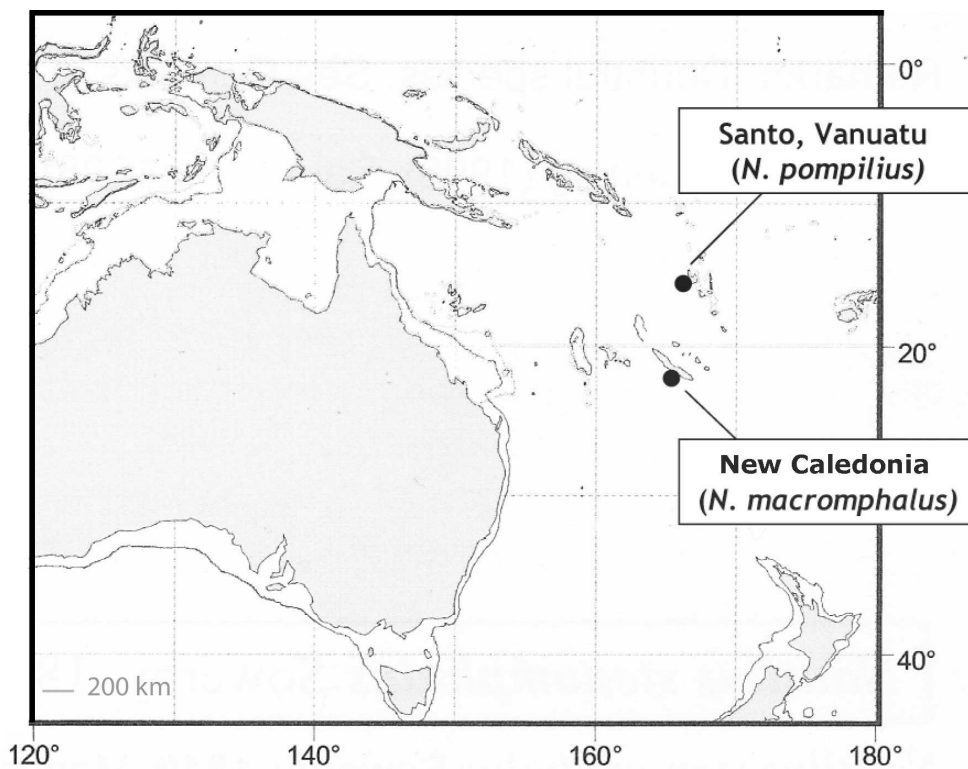
546 (black) and *N. macromphalus* from New Caledonia (white). Scale bars represent 1 standard

547 error.

548

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Figure 1.

554

