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Supplementary Material

The ascidian *Lissoclinum patella*, the patellamides and copper

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Sample collection

Intact specimen of *Lissoclinum patella* with sizes of 20-160 cm^2 and thicknesses of 5-15mm were collected in June and July 2022 (except for specimen 4 and 5, which were collected by Lars Behrendt in 2011), in and around the blue pools area at depths between 0.5m and 1.5m on Heron Island (23°26'04.4"S 151°55'21.1"E), two examples of collected specimen are shown in Figure S1. All specimen were transported in fresh seawater as quickly as possible to the Heron Island Research Station, where they were kept in shaded outdoor aquaria (less than 200 µMol Photons/m^2*sec) with continuous supply of fresh reef water. The collected animals were handled using sterile or sterilized equipment. All samples collected were kept at dry ice temperature or below until they were processed.

For the finely minced samples, a freshly cut 4x4cm piece of specimen was frozen and kept frozen until it was finely minced to be freeze-dried. For the cyanobacterial samples, the cyanobacteria and the cloacal liquid were collected from the specimen by cutting longitudinally through it and applying slight pressure, which causes the cyanobacteria to flow out. For the homogenate, a glass/teflon Potter Elvehjem homogenizer was used. To obtain only the water from the cloaca, the cyanobacteria were centrifuged off; for the filtered samples, a syringe filter (0.45 µm CA) was used.

In the sample "copper added" of specimen 3, a solution of 3x2ml 14.2 mM $Cu(SO_4)$ 6 H_2O was injected to one section of the living species, the other section was treated similarly with fresh water. The specimen was then kept in the aquarium before preparing it as described above (freezing, cutting, separating cloaka/Prochloron, homogenizing etc.).

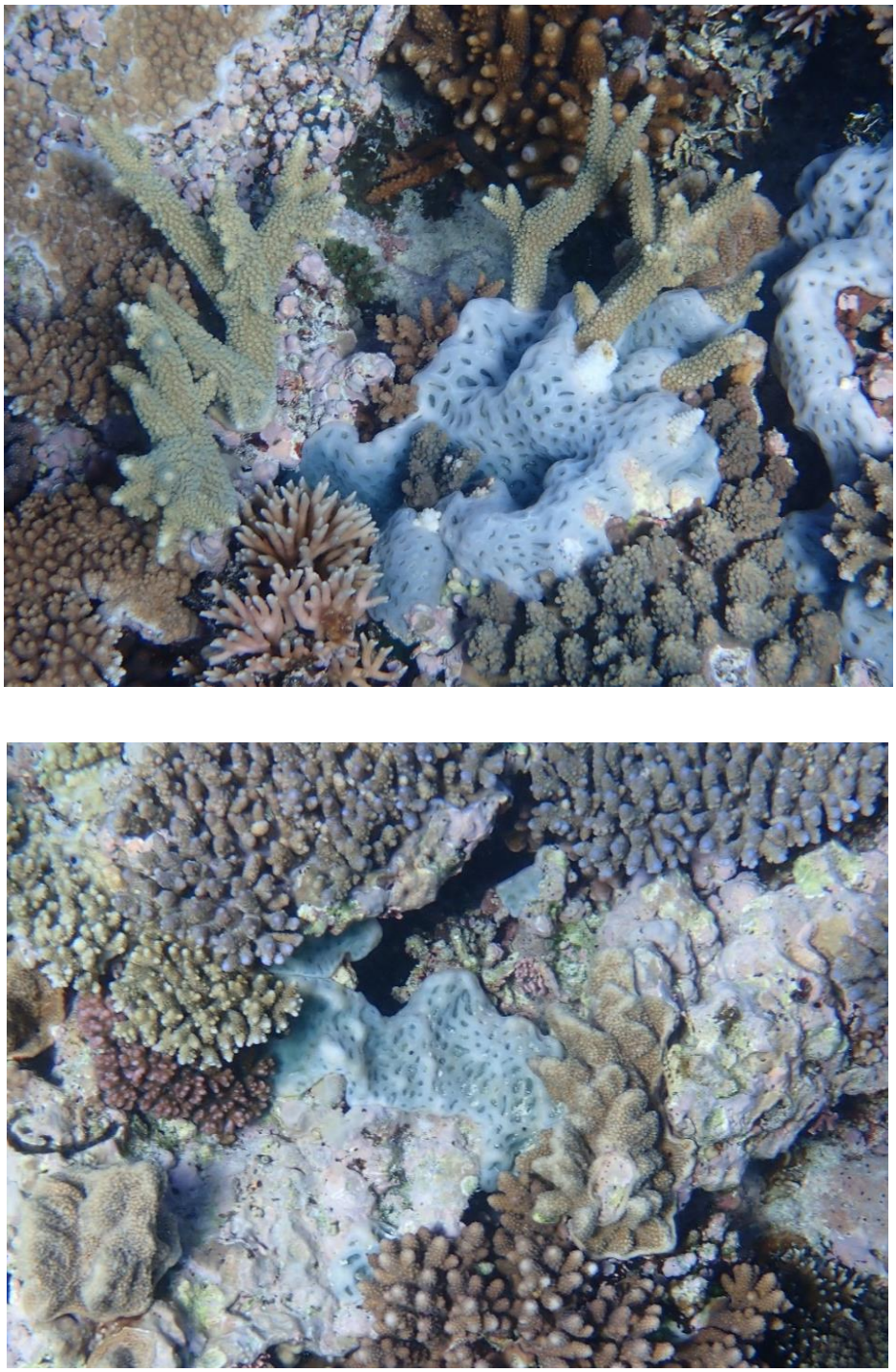

Figure S1. Collected specimen of *Lissoclinum patella.*

Sample preparation and determination of metal ion content

Digestions

For sample preparation, 2 ml of nitric acid (65 %, p.A., Neofroxx GmbH) and 125 µl of hydrogen peroxide (30 %, p.A., Merck KGaA) were added to the dried (20 mg – 70 mg) and liquid (250 µl) samples, respectively. All samples were prepared in duplicate. The resulting digestion suspensions were heated to 90 °C in a graphite block (Digiprep) for a period of one hour. Following complete digestion, samples were filled up to 10 ml with Milli-Q water.

For quality control, three different reference materials (CRM), BCR 060 Lagarosiphon Major, IAEA-336 Lichen and NCS ZC71001 Beef Liver (50 mg – 100 mg) were used, which were digested, like the samples in triplicates.

Because the samples of *Lissoclinum patella* were not completely dissolved after sample preparation, 3 ml of hydrochloric acid (32 %, p.A., Merck KGaA) were added to the suspensions and the samples again heated up 90 °C for a period of 0.5 h. Subsequent to the described procedure gelatinous streaks could be observed in the solution, which flocculated after filling the solution up to 10 ml with Milli-Q water. For this reason, *Lissoclinum patella* digestion solutions were filtered (0.45 µm) in advance of analysis.

Measurements

Copper concentrations were determined by ICP-OES (Agilent ICP-OES 720) at λ =327.395 nm. The relative standard deviation (RSD) of the measured digestion doublets ranges between 0.6 % and 7.4 %.

The recovery rates of the certified values of copper for the used CRM (measured by ICP-OES) ranges between 92.0 % and 98.2 %. The RSD of the triplet measurements ranges between 0.9 % and 6.2 %.

To verify the ICP-OES measurements, all samples were additionally measured by ICP-MS (Thermo Fisher iCAP TQ-e). All solutions were diluted ten times for analysis by adding Indium as internal standard. The RSD of the measured digestion doublets ranges between 0.6 % and 44.4 %.

The recovery rates of the certified values of copper for the used CRM (measured by ICP-MS) ranges between 79.8 % and 89.5 %. The RSD of the triplet measurements ranges between 2.0 % and 6.5 %.

Table S1. Metal ion concentrations with ICP-OES or ICP-MS, given in mg/kg (ppm) or mg/l for aqueous samples. Shown in this table are the water-loss corrected data before averaging (a simplified table is shown in the main text).

specimen			specimen 1	specimen 2	specimen 3		specimen 4
					no copper added	copper added	
whole specimen	OES	sample 1	0.27	0.94	-	-	0.34
		sample 2	0.26	0.84	-	-	0.27
	MS	sample 1	0.31	0.95	-	-	0.24
		sample 2	0.30	0.85	-	-	0.29
cyanobacteria (homogenized)	OES	sample 1	0.11	0.51	0.55	3.76	0.79
		sample 2	0.12	0.47	0.48	3.51	0.69
	MS	sample 1	0.14	0.52	0.60	3.68	-[a]
		sample 2	0.12	0.50	0.54	3.63	-[a]
water inside cloaka	OES	sample 1	0.05	0.06	0.13	0.53	-
		sample 2	0.05	0.06	0.13	0.50	-
	MS	sample 1	0.05	0.10	0.06	0.55	-
		sample 2	0.06	0.12	0.16	0.52	-

[a]Not sufficient substance was left for the comparison with ICP-MS

Table S2. Metal concentrations determined by ICP-OES, measured as mg/kg of the dissolved dry substance (DS) except for the aqueous samples (highlighted in blue), where it corresponds to the wet concentration in mg/l. For the reference materials used, the determined reference values are given to verify the reliability of the method.

	Ca g/kg DS	Cu mg/kg DS	Fe mg/kg DS	Mn mg/kg DS	Zn mg/kg DS
1 Specimen 1, whole, Sample 1	**180**	**2.21**	**19.3**	**1.78**	**6.02**
2 Specimen 1, whole, Sample 2	**136**	**2.03**	**22.7**	**1.54**	**6.75**
3 Specimen 2, whole, Sample 1	**213**	**5.57**	**28.5**	**1.82**	**5.69**
4 Specimen 2, whole, Sample 2Specimen	**201**	**4.99**	**41.5**	**2.35**	**6.54**
5 Specimen 1, cyano, Sample 1	**27.0**	**2.33**	**70.0**	**9.42**	**22.1**
6 Specimen 1, cyano, Sample 2	**26.0**	**2.43**	**75.0**	**9.43**	**21.8**
7 Specimen 2, cyano, Sample 1	**13.1**	**12.4**	**85.5**	**2.90**	**34.0**
8 Specimen 2, cyano, Sample 2	**12.9**	**11.3**	**73.7**	**2.72**	**31.3**
9 Specimen 3, cyano, no Cu, Sample	**18.7**	**13.1**	**106**	**3.47**	**34.1**
10 Specimen 3, cyano, no Cu, Sample	**20.3**	**11.3**	**88.4**	**3.26**	**30.2**
11 Specimen 3, cyano, +Cu, Sample 1	**17.5**	**81.8**	**183**	**5.45**	**48.3**
12 Specimen 3, cyano, +Cu, Sample	**21.2**	**76.4**	**181**	**5.36**	**46.8**
13 Specimen 3, cloaka, +Cu, Sample	**0.61**	**0.53**	**3.08**	**0.09**	**1.55**
14 Specimen 3, cloaka, +Cu, Sample	**0.61**	**0.50**	**2.08**	**0.09**	**1.54**
15 Specimen 3, cloaka, no Cu, Sample 1	**0.54**	**0.13**	**1.14**	**0.05**	**1.01**
16 Specimen 3, cloaka, no Cu, Sample 2	**0.56**	**0.13**	**1.43**	**0.05**	**1.03**
17 Specimen 2, cloaka, Sample 1	**0.72**	**0.06**	**0.82**	**0.10**	**1.13**
18 Specimen 2, cloaka, Sample 2	**0.72**	**0.06**	**1.29**	**0.11**	**1.14**
19 Specimen 1, cloaka, Sample 1	**0.62**	**0.05**	**1.34**	**0.05**	**0.46**
20 Specimen 1, cloaka, Sample 2	**0.66**	**0.05**	**1.02**	**0.06**	**0.43**
21 Specimen 4, cyano, Sample1	**30.5**	**8.7**	**433**	**54.5**	**36.1**
22 Specimen 4, whole, Sample 1	**241**	**1.5**	**36.1**	**2.35**	**5.58**
23 Specimen 4, whole, Sample 2	**240**	**1.2**	**31.0**	**2.30**	**5.01**
1 Specimen 1, whole, Sample 1					
Reference materials					
BCR 060 Lagarosiphon Major	**31.4**	**46.3**	**1908**	**187**	**311.0**
BCR 060 Lagarosiphon Major	**32.3**	**47.0**	**1638**	**1625**	**310.4**
BCR 060 Lagarosiphon Major	**33.8**	**53.0**	**1760**	**1737**	**334.0**
IAEA-336 Lichen	**2.66**	**3.29**	**300**	**68.4**	**31.2**
IAEA-336 Lichen	**2.56**	**3.35**	**321**	**70.4**	**31.5**
IAEA-336 Lichen	**2.49**	**3.29**	**306**	**68.4**	**31.0**
NCS ZC71001 Beef Liver	**0.19**	**90.2**	**348**	**9.67**	**201**
NCS ZC71001 Beef Liver	**0.20**	**93.1**	**380**	**10.2**	**207**
NCS ZC71001 Beef Liver	**0.19**	**86.6**	**373**	**9.77**	**199**

Reference values for the used reference materials					
reference values BCR 060	30.9	51.2	1190	1759	313
±		1.9		51	8
mean	32.5	48.8	1769	1183	318
SD	0.99	3.02	111	706	11.0
RSD %	3.03	6.19	6.25	59.7	3.44
recovery %	105	95.3	149	67.2	102
reference values IAEA 336	2.49	3.6	430	63	30.4
±	0.039	0.5	50	7	3.4
mean	2.57	3.31	309	69.1	31.26
SD	0.07	0.03	8.87	0.95	0.21
RSD %	2.64	0.86	2.87	1.37	0.68
recovery %	103	92.0	71.9	110	103
reference values NCS ZC71001	0.189	91.6	346	8.92	192
±	0.005	3.8	31	0.84	12
mean	0.19	89.95	367	9.87	203
SD	0.00	2.67	13.9	0.21	3.46
RSD %	2.28	2.97	3.78	2.15	1.71
recovery %	101	98.2	106	111	105

Table S3. Metal concentrations determined by ICP-MS, measured as mg/kg of the dissolved dry substance (DS), except for the aqueous samples (highlighted in blue), where it corresponds to the wet concentration in mg/l. For the reference materials used, the determined reference values are given to verify the reliability of the method. For some samples measured with ICP-MS, the deviation between the two samples was unusually high, thus the ICP-OES data seem to be more reliable.

	Cu mg/kg DS
1 Specimen 1, whole, Sample 1	**2.42**
2 Specimen 1, whole, Sample 2	**2.35**
3 Specimen 2, whole, Sample 1	**5.67**
4 Specimen 2, whole, Sample 2	**5.25**
5 Specimen 1, cyano, Sample 1	**2.92**
6 Specimen 1, cyano, Sample 2	**2.43**
7 Specimen 2, cyano, Sample 1	**12.6**
8 Specimen 2, cyano, Sample 2	**12.1**
9 Specimen 3, cyano, no Cu, Sample 1	**14.3**
10 Specimen 3, cyano, no Cu, Sample 2	**12.7**
11 Specimen 3, cyano, +Cu, Sample 1	**80.1**
12 Specimen 3, cyano, +Cu, Sample 2	**79.1**
13 Specimen 3, cloaka, +Cu, Sample 1	**0.55**
14 Specimen 3, cloaka, +Cu, Sample 2	**0.52**
15 Specimen 3, cloaka, no Cu, Sample 1	**0.06**
16 Specimen 3, cloaka, no Cu, Sample 2	**0.16**
17 Specimen 2, cloaka, Sample 1	**0.10**
18 Specimen 2, cloaka, Sample 2	**0.12**
19 Specimen 1, cloaka, Sample 1	**0.05**
20 Specimen 1, cloaka, Sample 2	**0.08**
21 Specimen 4, cyano, Sample1	**7.6**
22 Specimen 4, whole, Sample 1	**1.1**
23 Specimen 4, whole, Sample 2	**1.3**
reference Materials	
BCR 060 Lagarosiphon Major	**44.0**
BCR 060 Lagarosiphon Major	**44.0**
BCR 060 Lagarosiphon Major	**49.4**
IAEA-336 Lichen	**2.62**
IAEA-336 Lichen	**3.08**
IAEA-336 Lichen	**2.91**
NCS ZC71001 Beef Liver	**79.8**
NCS ZC71001 Beef Liver	**83.2**
NCS ZC71001 Beef Liver	**79.7**

reference values for the used reference materials	
reference value BCR 060	51.2
±	1.9
mean	45.8
SD	2.57
RSD %	5.62
recovery %	89.5
reference value IAEA 336	3.6
±	0.5
mean	2.87
SD	0.19
RSD %	6.54
recovery %	79.8
reference value NCS ZC71001	91.6
±	3.8
mean	80.9
SD	1.62
RSD %	2.00
recovery %	88.4

Table S4. Calculation of the copper content for the ICP-OES measurements (DS=dry substance, WS=wet substance).

Sample Type	**Cu mg/kg DS**	wet mass	dry mass	remaining weight	factor	**Cu mg/kg WS**
1 Specimen 1 (finely minced)	**2.21**	3.686	0.466	12.63%	7.916	0.28
2 Specimen 1 (finely minced)	**2.03**	3.686	0.466	12.63%	7.916	0.26
3 Specimen 2 (big slices)	**5.57**	1.289	0.217	16.82%	5.944	0.94
4 Specimen 2 (big slices)	**4.99**	1.289	0.217	16.82%	5.944	0.84
5 Prochloron Homogenate Specimen 1	**2.33**	1.375	0.067	4.85%	20.621	0.11
6 Prochloron Homogenate Specimen 1	**2.43**	1.375	0.067	4.85%	20.621	0.12
7 Prochloron Homogenate Specimen 2	**12.4**	2.066	0.086	4.15%	24.084	0.51
8 Prochloron Homogenate Specimen 2	**11.3**	2.066	0.086	4.15%	24.084	0.47
9 Prochloron Homogenate Specimen 3 No Copper Added	**13.1**	1.708	0.072	4.23%	23.624	0.55
10 Prochloron Homogenate Specimen 3 No Copper Added	**11.3**	1.708	0.072	4.23%	23.624	0.48
11 Prochloron Homogenate Specimen 3 Copper Added (3x 2mL of 14.2mM CuSO4x6H2O)	**81.8**	1.883	0.087	4.59%	21.772	3.76
12 Prochloron Homogenate Specimen 3 Copper Added (3x 2mL of 14.2mM CuSO4x6H2O)	**76.4**	1.883	0.087	4.59%	21.772	3.51
13 Water from Cloaka Specimen 3 Copper Added (3x 2mL of 14.2mM CuSO4x6H2O)	**0.53**	0.600	0.600	100.00%	1.000	0.53
14 Water from Cloaka Specimen 3 Copper Added (3x 2mL of 14.2mM CuSO4x6H2O)	**0.50**	0.600	0.600	100.00%	1.000	0.50
15 Water from Cloaka Specimen 3 No Copper Added	**0.13**	0.600	0.600	100.00%	1.000	0.13
16 Water from Cloaka Specimen 3 No Copper Added	**0.13**	0.600	0.600	100.00%	1.000	0.13
17 Water from Cloaka Specimen 1/2 Filtered	**0.06**	0.600	0.600	100.00%	1.000	0.06
18 Water from Cloaka Specimen 1/2 Filtered	**0.06**	0.600	0.600	100.00%	1.000	0.06
19 Water from Cloaka Specimen 1/2 Unfiltered	**0.05**	0.600	0.600	100.00%	1.000	0.05
20 Water from Cloaka Specimen 1/2 Unfiltered	**0.05**	0.600	0.600	100.00%	1.000	0.05
21 Prochloron Homogenate Specimen 5	**8.7**	0.321	0.029	9.10%	10.993	0.79
22 Specimen 4, whole	**1.5**	0.281	0.063	22.55%	4.435	0.34
23 Specimen 4, whole	**1.2**	0.281	0.063	22.55%	4.435	0.27

Table S5. Calculation of the Copper Content for the ICP-MS measurements (DS=dry substance, WS=wet substance).

Sample Type	Cu mg/kg DS	wet mass	dry mass	remaining weight	factor	Cu mg/kg WS
1 Specimen 1 (finely minced)	**2.42**	3.686	0.466	12.63%	7.916	**0.31**
2 Specimen 1 (finely minced)	**2.35**	3.686	0.466	12.63%	7.916	**0.30**
3 Specimen 2 (big slices)	**5.67**	1.289	0.217	16.82%	5.944	**0.95**
4 Specimen 2 (big slices)	**5.25**	1.289	0.217	16.82%	5.944	**0.88**
5 Prochloron Homogenate Specimen 1	**2.92**	1.375	0.067	4.85%	20.621	**0.14**
6 Prochloron Homogenate Specimen 1	**2.43**	1.375	0.067	4.85%	20.621	**0.12**
7 Prochloron Homogenate Specimen 2	**12.6**	2.066	0.086	4.15%	24.084	**0.52**
8 Prochloron Homogenate Specimen 2	**12.1**	2.066	0.086	4.15%	24.084	**0.50**
9 Prochloron Homogenate Specimen 3 No Copper Added	**14.3**	1.708	0.072	4.23%	23.624	**0.60**
10 Prochloron Homogenate Specimen 3 No Copper Added	**12.7**	1.708	0.072	4.23%	23.624	**0.54**
11 Prochloron Homogenate Specimen 3 Copper Added (3x 2mL of 14.2mM CuSO4x6H2O)	**80.1**	1.883	0.087	4.59%	21.772	**3.68**
12 Prochloron Homogenate Specimen 3 Copper Added (3x 2mL of 14.2mM CuSO4x6H2O)	**79.1**	1.883	0.087	4.59%	21.772	**3.63**
13 Water from Cloaka Specimen 3 Copper Added (3x 2mL of 14.2mM CuSO4x6H2O)Specimen	**0.55**	0.600	0.600	100.00%	1.000	**0.55**
14 Water from Cloaka Specimen 3 Copper Added (3x 2mL of 14.2mM CuSO4x6H2O)Specimen	**0.52**	0.600	0.600	100.00%	1.000	**0.52**
15 Water from Cloaka Specimen 3 No Copper AddedSpecimen	**0.06**	0.600	0.600	100.00%	1.000	**0.06**
16 Water from Cloaka Specimen 3 No Copper AddedSpecimen	**0.16**	0.600	0.600	100.00%	1.000	**0.16**
17 Water from Cloaka Specimen 1/2 FilteredSpecimen	**0.10**	0.600	0.600	100.00%	1.000	**0.10**
18 Water from Cloaka Specimen 1/2 FilteredSpecimen	**0.12**	0.600	0.600	100.00%	1.000	**0.12**
19 Water from Cloaka Specimen 1/2 UnfilteredSpecimen	**0.05**	0.600	0.600	100.00%	1.000	**0.05**
20 Water from Cloaka Specimen 1/2 UnfilteredSpecimen	**0.08**	0.600	0.600	100.00%	1.000	**0.08**
21 Prochloron Homogenate Lars Specimen	**7.6**	0.321	0.029	9.10%	10.993	**0.69**

22 Specimen LarsSpecimen	**1.1**	0.281	0.063	22.55%	4.435	**0.24**
23 Specimen LarsSpecimen	**1.3**	0.281	0.063	22.55%	4.435	**0.29**

Table S6. Complex stabilities for different natural patellamide derivatives reported in literature (adapted from [1, 2])

metal ion	cyclic peptide	method	K_1	K_2	reference
Cu^{II}	Patellamide A	CD	$2.00x10^4$	$7.76x10^2$	[3], [4]
		MS	$3.31x10^4$	$1.00x10^4$	[3]
Cu^{II}	Patellamide B	CD	$3.02x10^5$	$2.29x10^2$	[4]
Cu^{II}	Patellamide C	CD	$6.76x10^4$		[3]
		MS	$6.31x10^4$	$6.03x10^3$	[4]
Cu^{II}	Patellamide E [a]	CD	$1.51x10^4$		[3]
Zn^{II}	Ascidiacyclamide	NMR, CD	$1.00x10^3$		[5]
Zn^{II}	Patellamide A	CD	$3.02x10^4$	$1.00x10^3$	[3]
		MS	$2.82x10^3$	$3.89x10^3$	[3]
Zn^{II}	Patellamide B	CD	$3.02x10^4$	$1.91x10^1$	[4]
Zn^{II}	Patellamide C	CD	$1.78x10^4$	$8.13x10^2$	[3]
		MS	$2.40x10^3$	$2.57x10^3$	[3]
Zn^{II}	Patellamide E [a]	CD	$7.94x10^4$	$2.00x10^1$	[4]
Cu^{II}	Lissoclinamide 9	CD	$1.41x10^4$	$1.26x10^2$	[3]
Cu^{II}	H4pat1	ITC	$1.71x10^6$		[1]
Cu^{II}	H4pat2	ITC	$4.03x10^4$		[1]
Cu^{II}	H4pat3	ITC	$2.27x10^5$		[1]
Cu^{II}	H4pat4	ITC	$1.43x10^5$		[1]
Cu^{II}	H4pat5	ITC	$1.50x10^5$		[1]

[a] In contrast to all other published data, for patellamide E the Zn^{2+} stability is slightly larger than that of Cu^{2+}. In view of the small structural changes, this is not as expected. Moreover, a careful study of the original publications indicates that there are problems with data analyses (specifically for these experiments but possibly also for others based on CD spectroscopy). This also appears from the large differences between K1 and K2, when generally it has been observed that binding of the second metal ion is cooperative (see also main text).

Table S7. Literature data for the concentration of copper in seawater at various locations, reported over the last two decades and determined by a range of different methods.

Year	Place	range [ppb]	average [ppb]	ref
1979	Global	0.15	0.15	[6]
1983	Northwest Atlantic	0.13-0.19	0.16	[7]
1985	North-East Atlantic, deep open water[a]	0.073-0.094	*0.09*	[8]
1986	Great Barrier Reef (Heron Island)	0.12-0.24	0.14	[9]
1988	Northeast Pacific, deep open water[a]	0.038	*0.038*	[10]
1994	Heron Island[b]	0.08-27	*13.5*	[11]
1998	Global	0.04-10	not rep.	[12]
2001	Global	0.25	0.25	[13]
2011	Red Sea[a]	2.08-5.23	*3.244*	[14]
2014	Northern Pacific Ocean, (one in Indian Ocean)	0.0378-0.29	0.07	[15]
2018	Global Values	0.5-3.18	0.5	[16]
average			0.21	

[a]This value was not used for the average, since the sampling location/conditions cannot be assumed to be similar to those of the Great Barrier Reef
[b]This value was not used for the average, see text

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