Supplementary 1. *Sterol identification, mass spectra and retention times*

Analysis by GC-MS (underivatized and as TMS ethers) allowed the identification of the main sterol constituents (Fig. S2, S3). However, analysis by GCxGC-TOFMS showed an increased signal-to-noise ratio particularly for the ethanol samples, where some contaminants or oxosterols had previously concealed the presence of smaller, in the first dimension partially co-eluting compounds in the extracts. It was thus further used for identification and semi-quantification. In order to increase the separation efficiency on the polar, secondary column, underivatized sterol fractions rather than TMS ethers were used.

Sterols in the sponge extracts were identified based on GC retention times (Primary, or 1st column: Rt_1; Secondary, or 2nd column: Rt_2) and mass spectra, *via* comparison to library spectra or previously published spectra (as referenced below). 26 different sterols were detected in the three sponges (Table S1). Most sterols could be identified based on their mass spectra, such as the sterols from the cholestane series with 27 carbon atoms (**1-4**, Fig. 1, Table S1), of which cholesta-5,22-dien-3β-ol (**1**), 5α-cholest-22-en-3β-ol (**2**), cholest-5-en-3β-ol (**3**) and 5α-cholestan-3β-ol (**4**). 5α-cholest-7-en-3β-ol (**7**) showed a very similar mass spectrum to (**3**), but a higher Rt_1, which indicates that the double bond was present at the 7-position (Gerst et al., 1997). The Rt_2 of sterols were around 3.5 to 3.8 seconds, and generally around 0.5 seconds lower than the corresponding 3-oxosterols, with a ketone group instead of the hydroxyl group at position 3 (Fig. 2), a valuable feature in distinguishing 3-oxo and 3-hydroxy compounds. A number of sterols from the ergostane series (28 carbon atoms) could be identified, with 5α-ergostan-3β-ol (**13**) as the fully saturated representative, and with a saturated side chain but unsaturation in the ring system (**11**, **17**). The latter showed a molecular ion ($M^{+\cdot}$) at *m/z* 400, indicating one unsaturation, and a fragment at *m/z* 213 produced after loss of the D-ring, the side chain and H_2O, which indicates an unsaturated ring system. As with the cholestane series, the location of unsaturation was determined from the elution order as specified by Gerst et al. (1997): the sterol with the double bond between carbon 8 and 14 [$=\Delta^{8(14)}$] eluted first, followed by Δ^5, $\Delta^{8(9)}$ and then Δ^7 sterols. Ergostane-type sterols with one double bond on the side chain (SC) (**6**, **12**) showed a molecular ion at *m/z* 400. 5α-ergost-22-en-3β-ol (**6**) additionally showed strong peaks for the fragments *m/z* 273, 257 and 213

(M^{+}·-SC-42-H_2O), while the base peak of 5α-ergost-24(24^1)-en-3β-ol was observed at 316, a result of a McLafferty fragmentation and loss of part of the side chain. Similarly, C_{28} sterols with two unsaturations could be determined: the mass spectrum of ergosta-5,22-dien-3β-ol showed the characteristic fragments 271, 255 and 213 (M-SC-42-H_2O), while the base peak in the mass spectrum of ergosta-5,24(24^1)-dien-3β-ol was at *m/z* 314, diagnostic for Δ^{24} sterols. A triunsaturated sterol could also be resolved by GC×GC, tentatively assigned as ergostatrienol, with all double bonds located in the A, B and C rings in the ring system (numbering see Fig. 1B), as such presumably $\Delta^{5,7,9(11)}$. **10** was only detected in the ethanol of Agelas sp. MF1, and could thus be an artefact or a contaminant. The mass spectrum indicates an unsaturated core, and a cyclic side chain, and is thus possibly 23,24^1-cycloergost-5-en-3β-ol. A large number of different sterols from the stigmastane series were also identified: 5α-stigmastan-3β-ol (**24**) was the fully saturated sterol, while sterols with one unsaturation in the ring system (stigmast-5-en-3β-ol, **19**, 5α-stigmast-8-en-3β-ol, **25**, and 5α-stigmast-7-en-3β-ol, **26**) were also detected. Due to the similarity of their mass spectra, the positions of the double bond were assigned based on retention times, with Rt_1 of $\Delta^{8(14)} < \Delta^5 < \Delta^{8(9)} < \Delta^7$. C_{29} sterols with one unsaturation in the side chain were are also detected and included 5α-stigmast-22-en-3β-ol (**16**), and two isomers of 5α-stigmast-24(24^1)-en-3β-ol (M^{+}· *m/z* 414, base peak *m/z* 316), which were identified as the 24(*E*)- (**21**) and the 24(*Z*)- (**24**) isomers based on retention times, which are greater for the latter. It has to be noted that, in the first dimension, we observed co-elution of the 24(*E*)-isomer with stigmasta-5,24(24^1)-dien-3β-ol (**22** M^{+}· *m/z* 412, base peak *m/z* 314), while the compounds could be resolved in the second dimension. Other isomers with two unsaturations included stigmasta-5,22-dien-3β-ol (**15**), 5α-stigmast-7,22-dien-3β-ol (**20**). An unusual sterol that was tentatively identified in *Ecionemia* sp. SS1 (ethanol and lyophilized) by spectral matching was 23,24^1-cyclostigmasta-5-en-3β-ol (**18**, = dihydrocalysterol, Li et al. 1982). Identification of this sterol was based on its molecular ion of *m/z* 412 of low abundance, the presence of an ion of *m/z* of 314, m/z 271 and *m/z* 213 and comparison to the spectra published by Li et al. (1982). An unusually early eluting C_{29} sterol (**9**) with an M+· of *m/z* 414, and a strong peak at *m/z* 213, were also detected and tentatively assigned as a C_{29} sterol with one unsaturation in the core.

Table S1. Identified sterols in the compounds, retention times and *m/z* used for identification. 'Compound nr.' indicates the number of the compound used in Fig. 1, 'sterol name' the name according to IUPAC nomenclature (IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), 1989), 'Rt_1' the retention time in the first dimension in seconds, 'Rt_2' the retention time in the second dimension in seconds, with a 5 second modulation period, and MS data the fragments that were observed and used for identification.

Sterol nr. (see Fig. 2)	**Sterol name**	**[$M^{+}\cdot$]**	Rt_1	Rt_2	**MS data (typical fragments)**
1	Cholesta-5,22-dien-3β-ol	384	4905	3.52	384, 366, 351, 300, 285, 271, 255, 229, 213, 199
2	5α-Cholest-22-en-3β-ol	386	4920	3.49	386, 371, 353, 302, 287, 273, 257, 245, 232, 215, 201
3	Cholest-5-en-3β-ol	386	4960	3.56	386, 368, 353, 301, 275, 255, 231, 213, 199
4	5α-Cholestan-3β-ol	388	4970	3.50	388, 373, 355, 262, 233, 215
5	Ergosta-5,22-dien-3β-ol	398	5010	3.57	398, 365, 355, 337, 300, 271, 255, 213, 199
6	5α-Ergost-22-en-3β-ol	400	5025	3.54	400, 339, 316, 302, 287, 273, 257, 242, 215
7	5α-Cholest-7-en-3β-ol	386	5025	3.66	386, 371, 353, 301, 273, 255, 231, 213, 199
8	5α-Ergosta-5,24(24^1)-dien-3β-ol	398	5080	3.71	398, 314, 299, 281,271, 255, 229, 213, 199
9	C_{29}Δ-Sterol	414	5070	3.83	414, 399, 382, 381, 301, 283, 273, 269, 213
10	23,24^1-Cycloergost-5-en-3β-ol	398	5080	3.73	398, 383, 271, 255, 229, 213
11	Ergost-5-en-3β-ol	400	5090	3.62	400, 382, 367, 340, 327, 315, 289, 255, 231, 213, 199
12	5α-Ergost-24(24^1)-en-3β-ol	400	5095	3.70	400, 385, 367, 316, 301, 283, 273, 255, 233, 215, 201
13	5α-Ergostan-3β-ol	402	5110	3.66	402, 387, 369, 327, 299, 276, 233, 215
14	Ergostatrien-3β-ol	396	5130	3.86	408, 390, 375, 277, 267, 251, 235, 225, 209
15	Stigmasta-5,22-dien-3β-ol	412	5125	3.61	412, 397, 379, 300, 271, 255, 229, 213
16	5α-Stigmast-22-en-3β-ol	414	5145	3.59	414, 257, 215
17	5α-Ergost-7-en-3β-ol	400	5165	3.81	400, 357, 327, 273, 255, 213
18	23,24^1-Cyclostigmast-5-en-3β-ol	412	5185	3.72	412, 394, 379, 352, 338, 327, 314, 301, 281, 271, 255, 231, 213, 199
19	Stigmast-5-en-3β-ol	414	5195	3.80	414, 396, 381, 367, 339, 329, 303, 273, 255, 241, 231, 213, 199
20	5α-Stigmasta-7,22-dien-3β-ol	412	5200	3.85	412, 369, 351, 300, 271, 255, 229, 213
21	(E)-Stigmast-24(24^1)-en-3β-ol	414	5210	3.77	414, 399, 381, 316, 301, 283, 273, 233, 215, 203
22	Stigmasta-5,24($24^{1)}$-dien-3β-ol	412	5210	3.85	412, 397, 379, 314, 299, 281, 271, 255, 253, 229, 213, 211, 199
23	5α-Stigmastan-3β-ol	416	5215	3.67	416, 401, 383, 355, 316, 290, 248, 233, 215
24	(Z)-Stigmast-24(24^1)-en-3β-ol	414	5220	3.80	414, 399, 381, 316, 301, 283, 273, 233, 215, 203, 201, 199
25	5α-Stigmast-8-en-3β-ol	414	5225	3.73	414, 396, 381, 355, 329, 315, 303, 267, 255, 231, 213, 199
26	5α-Stigmast-7-en-3ß-ol	414	5270	3.95	414, 399, 381, 273, 255, 231, 213, 201, 199

References

Gerst N, Ruan B, Pang J, Wilson WK, Schroepfer GJ (1997) An updated look at the analysis of unsaturated C_{27} sterols by gas chromatography and mass spectrometry. *Journal of Lipid Research* **38**, 1685–1701.

IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN) (1989) The nomenclature of steroids. *European Journal of Biochemistry* **186**, 429–458.

Li LN, Li HT, Lang RW, Itoh T, Sica D, Djerassi C (1982) Minor and trace sterols in marine invertebrates. 31. Isolation and structure elucidation of 23H-isocalysterol, a naturally occurring cyclopropene. Some comparative observations on the course of hydrogenolytic ring opening of steroidal cyclopropenes and cyclopropanes. *Journal of the American Chemical Society* **104**, 6726–6732.

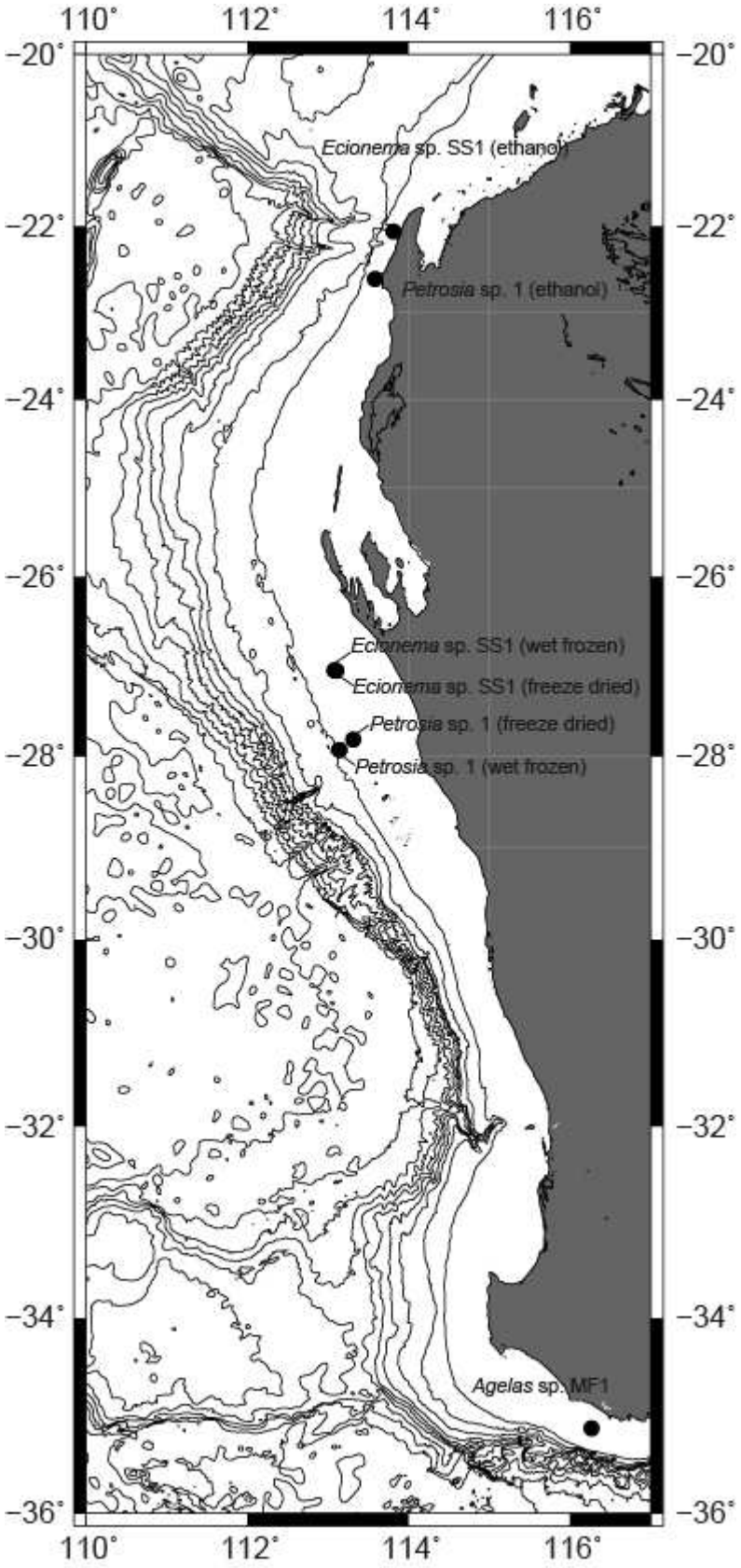

Figure S1. Map showing the sampling locations for all 9 sponge specimens. Bathymetry is shown with 500 m contour lines.

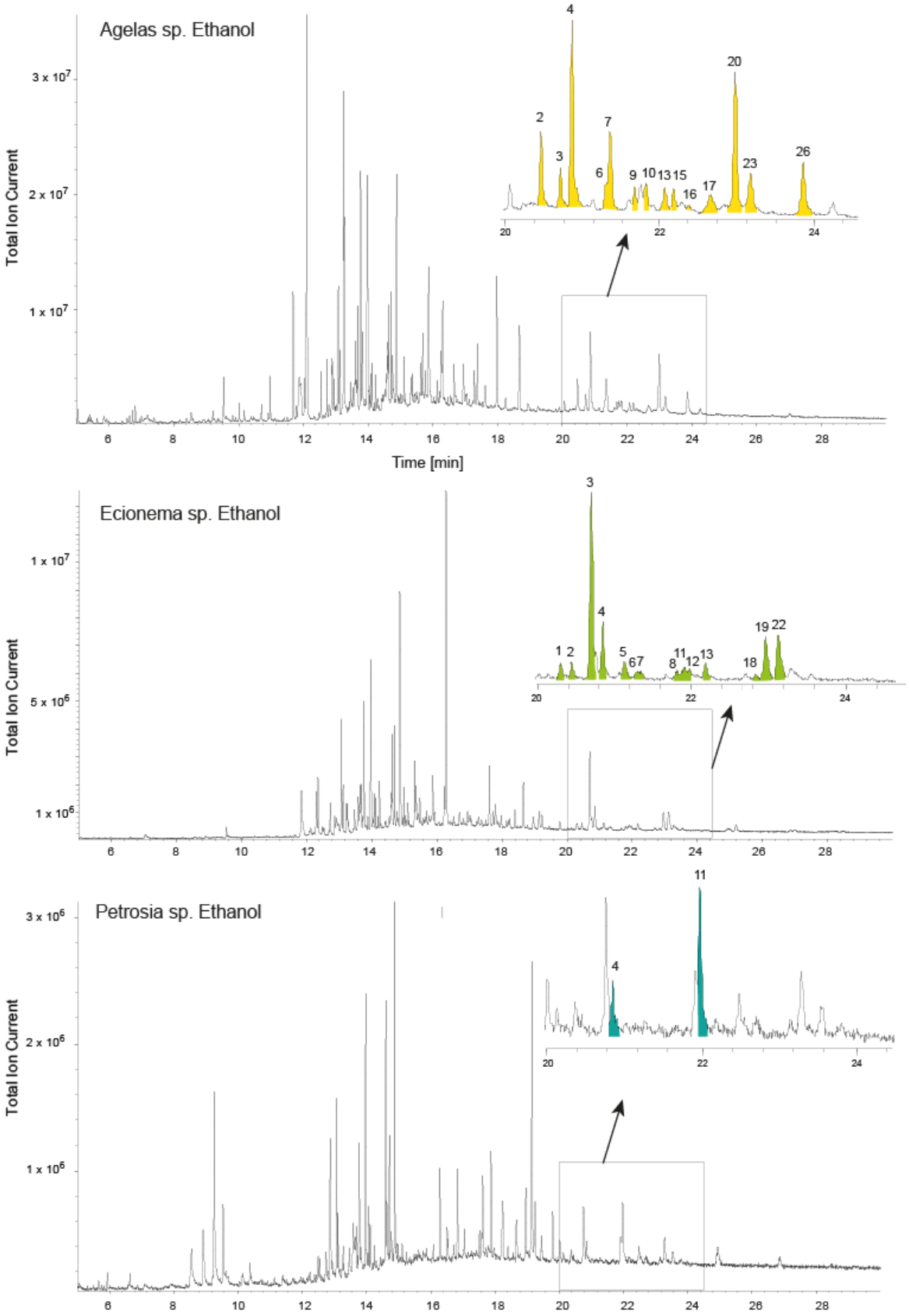

Figure S2. Chromatograms of the underivatized polar fraction obtained by GC-MS. A – *Agelas* sp. MF1, B – *Ecionemia* sp. SS1, C – *Petrosia* sp. 1.

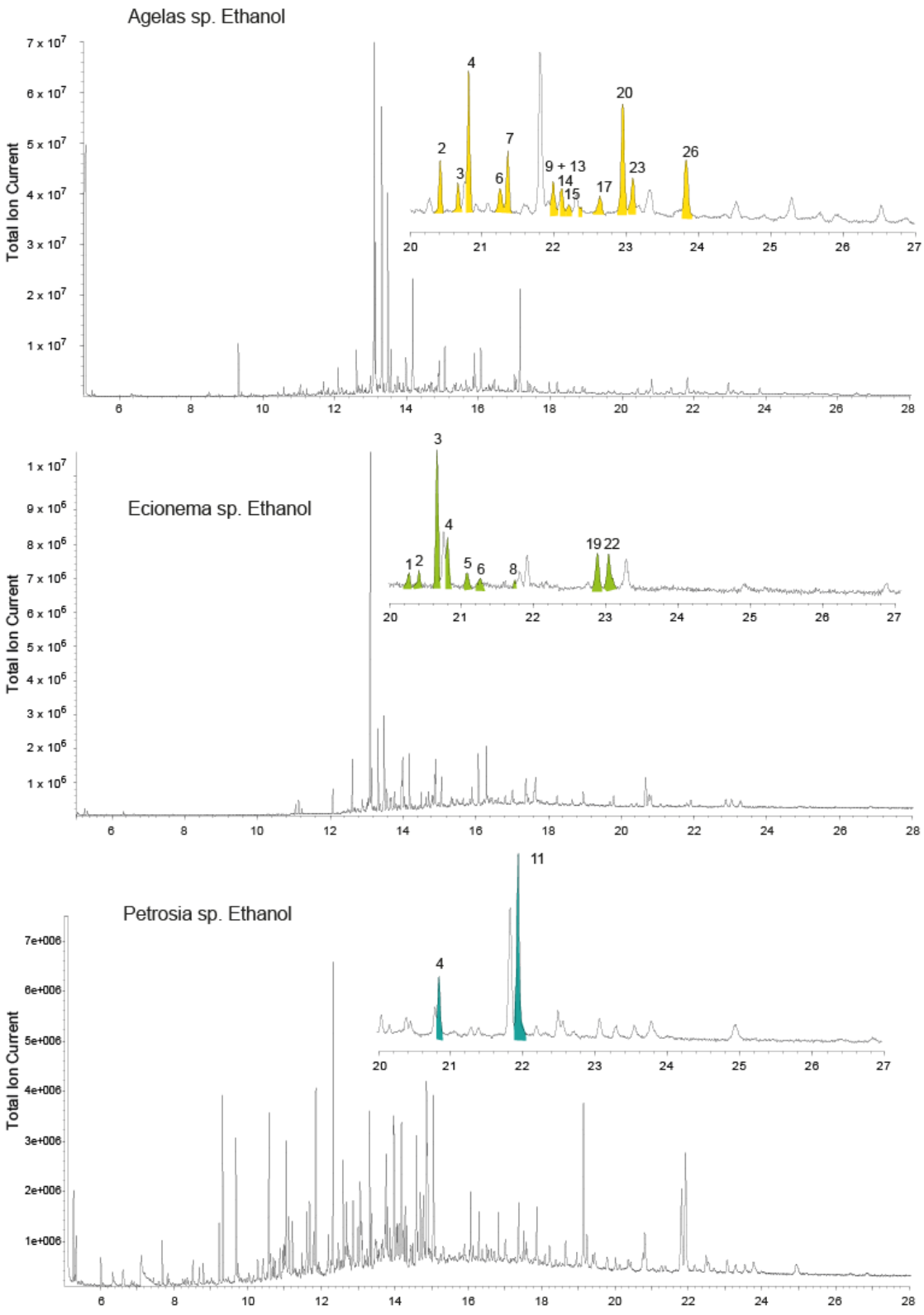

Figure S3. Chromatograms of the BSTFA-derivatized polar fraction (TMS ethers) obtained by GC-MS. A – *Agelas* sp. MF1, B – *Ecionemia* sp. SS1, C – *Petrosia* sp. 1.

Figure S4.

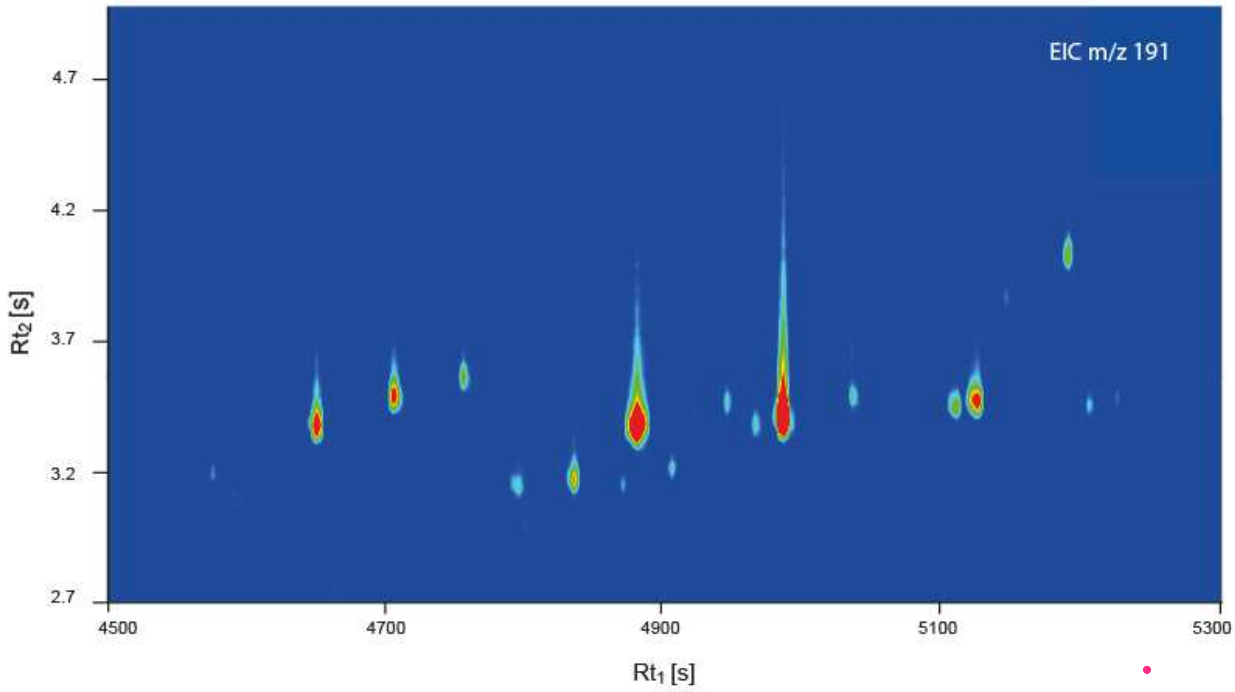

Figure S4. Extracted ion current of *m/z* 191 for the saponified biomass showing possible triterpenoid compounds which were not present in any of the extracts.