

II (A), C II, 2014), (l, 2013), A, C (D l, 2011; l, 2011; l, 2013), C, I A (I A), l, 2010; l, 2010), A B l (2013), 18 I A, 18 I A, A, D I A, D I A

Materials and methods

Sample collection

C BA 15-10- (112°20'E, 16°50') C, A, 5, 2, 3, 20 I A (I A), I A (I A) <15 . A

80 °C, I A (I A), (3 (0.22 μ) E 20 I A (I A), -80 °C

Nucleic acid extraction and cDNA synthesis

D I A, I A, A D I A/ I A, E, F, I A, A, D I A, I A, D I A, A F, D I A, I A, C, D 2000 (E AB B), D I A, -20 °C, -80 °C

PCR, cloning and sequencing

I A (I A) (I A), l, 1990; l, 1999; B & I A, 2000; D I A, 2001; & I A, 2012; l, 2012). D I A A II, (D l, 2011), C . C : 5 94 °C, 35 94 °C 1, 1) (I A), 72 °C 1 . F 72 °C 10 . C (I A) 1.2% (/) (C B , C) C 2.1- C E . I A (I A). B 13F/ C A E 2

1. PCR primers and results

Primer	Length of PCR product (bp)	No. of sequenced clones		Annealing temperature (°C)	Reference
		No. of fungal inserts	No. of sponge inserts		
SSU					
nu-SSU-0817-5/nu-SSU-1196-3	379	57	35	54	Borneman & Hartin (2000)
nu-SSU-0817-5/nu-SSU-1536-3	719	17	37	56	
EF4/fung5	558	283	5	55	Smit <i>et al.</i> (1999)
EF4/EF3	1400	4	37	53	
EukA/EukB	1700	0	49	55	Díez <i>et al.</i> (2001)
ITS					
ITS1/ITS4	630	0	34	55	White <i>et al.</i> (1990)
ITS1/ITS2	300	0	29	56	
LSU					
LROR/LR3	600	0	10	50	Poter & Golding (2012)
LROR/LR5	950	0	17	48	Schoch <i>et al.</i> (2012)

Statistical and phylogenetic analysis

A 1.29
 (L, 2009). A
 115 (L, 2013).
 E. B.
 (C, L, 2010). C
 18 A
 (80%).
 3% 1.29.
 E. B.
 F AC E. A
 (D) A A
 AB2010. F (. 550)
 B A
 C B ; // .
 /B .) E A 5.1,
 C A E A 5.1 (L,
 2011).
 E A 5.1 .
 E A 5.1 . (100 -
)
 18 A

Nucleotide sequence accession numbers

B
 245896– 245921.

Results

Feasibility of different primers

A
 A 1, C -
 0 / 5, 0 / 3, E A/
 E B,
 EF4/ 5
 283 18
 A (88)
 -0817-5/ -1196-3, -0817-5/ -
 1536-3, EF4/EF3
 , EF4/
 5
 F , EF4/ 5
 18 A
 D A A
 D A

Richness and distribution pattern of sponge-associated fungi

A 2,
 (> 90%) A
 C 1,
 ()
),
 F AC,

(D), A, F, D
 (F. 1.),

2. Sequencing results and statistical analysis

	SW	TD	TR	XD	XR
No. of sequences*	88	49	53	44	49
No. OTUs†	15	10	9	7	4
Goods_coverage‡	0.955	0.918	0.925	0.932	0.980
Chao1§	17	12	12	9	4
Shannon¶	3.323	1.849	2.227	1.615	1.627
Simpson¶	0.875	0.529	0.703	0.502	0.647

TD, *Theonella swinhoei* DNA-derived library; TR, *T. swinhoei* cDNA-derived library; XD, *Xestospongia testudinaria* DNA-derived library; XR, *X. testudinaria* cDNA-derived library; SW, seawater cDNA-derived library.

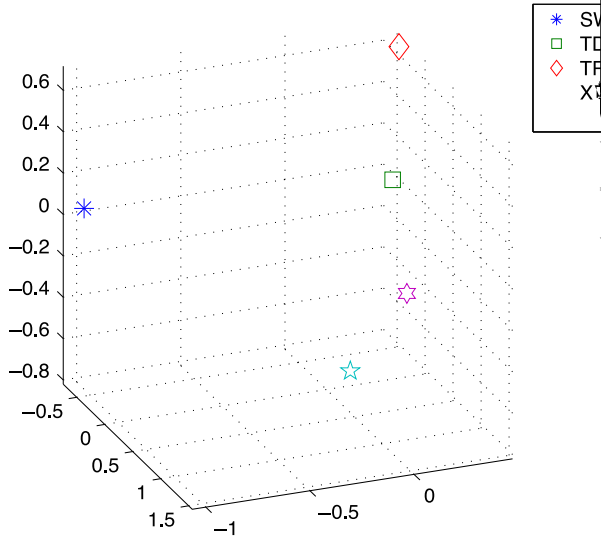
*Only high quality sequences (283 out of 359) were showed and submitted to subsequent analysis.

†The observed OTU numbers which were close/equal to Chao1 indices suggested the sequencing effort was sufficient to generate reliable diversity information, which was supported by Goods_coverage (> 90%) as well.

‡Goods_coverage indicated the coverage of each library.

§Chao1, nonparametric richness estimator, indicated the richness of each dataset.

¶Shannon and Simpson are the diversity index. High Shannon/Simpson values imply high diversity.



... A ...
 ... D¹ A ...
 ... D¹ A ...
 ... A ...
 ... 3 ...
 ... D¹ A ...
 ... : (1) ...
 ... D¹ A ... ; (2) ...
 ... D¹ A ...
 ... (3) ...
 ... (2). F ...
 ... : (1) ...
 ... 8, 21 ...
 ... ; (2) ...
 ... (2) ...
 ... ; (3) ...
 ... D¹ A ...
 ... 18, 10, ...
 ... D¹ A ... ; (4) ...
 ... 19, 24; (5) ...
 ... B ...
 ... D¹ A ...

taxonomy assignments and phylogenetic analysis

... A ... B ... B1 ...
 ... A ...
 ... (15/26)

3. OTU distribution and BLAST results

OTU	XD	XR	TR	TD	SW	Next relative	ACCN	Identity (%)
OTU8	0	0	0	0	18	<i>Balansia henningsiana</i>	AY545727	99
OTU21	0	0	0	0	16	<i>Eupenicillium limosum</i>	EF411061	98
OTU1	0	0	0	0	14	<i>Peziza proteana</i>	AY544703	97
OTU17	1	0	0	0	9	<i>Balansia henningsiana</i>	AY545727	98
OTU18	1	11	7	1	8	<i>Mycosphaerella punctiformis</i>	DQ471017	100
OTU10	1	14	12	3	6	<i>Alternaria alternata</i>	DQ678031	99
OTU7	0	0	0	0	6	<i>Peziza proteana</i>	AY544703	97
OTU22	0	0	0	0	4	<i>Botryotinia fuckeliana</i>	AY544695	98
OTU2	26	23	25	33	1	<i>Aspergillus fumigatus</i>	AB008401	99
OTU23	0	0	0	0	1	<i>Neofabraea malicorticis</i>	AY544706	95
OTU4	0	0	0	0	1	<i>Xylaria hypoxylon</i>	AY544692	97
OTU14	0	0	0	0	1	<i>Aureobasidium pullulans</i>	DQ471004	94
OTU20	0	0	0	0	1	<i>Cryptococcus gastricus</i>	DQ645513	97
OTU5	0	0	0	0	1	<i>Eutypa lata</i>	DQ836896	94
OTU9	0	0	0	0	1	<i>Catenomyces</i> sp.	AY635830	99
OTU11	0	0	1	0	0	<i>Pleospora herbarum</i>	DQ767648	96
OTU12	0	0	2	0	0	<i>Catenomyces</i> sp.	AY635830	99
OTU13	0	0	1	1	0	<i>Orbilia auricolor</i>	DQ471001	97
OTU15	1	0	0	3	0	<i>Tricholoma aestuans</i>	AY757267	93
OTU16	0	0	1	1	0	<i>Petriella setifera</i>	DQ471020	98
OTU19	9	0	0	4	0	<i>Cladosporium</i> sp.	AY016351	99
OTU24	4	0	0	0	0	<i>Cochliobolus heterostrophus</i>	AY544727	99
OTU25	1	1	0	0	0	<i>Dothiora cannabinae</i>	DQ479933	99
OTU26	0	0	1	1	0	<i>Meria laricis</i>	DQ471002	94
OTU3	0	0	0	1	0	<i>Cladosporium herbarum</i>	DQ678022	98
OTU6	0	0	3	1	0	<i>Chaetomium globosum</i>	AY545725	99

XD, *Xestospongia testudinaria* DNA-derived library; XR, *X. testudinaria* cDNA-derived library; TD, *Theonella swinhoei* DNA-derived library; TR, *T. swinhoei* cDNA-derived library; SW, seawater cDNA-derived library.

(8, 21) (2). D
 , 148
 77.3%
 53%
 D A
 (F . 2)
 2,
 A. II
 ; 10,
 D A
 (= 95), 24

I B I. (2013),
 A A
 A
 (I, 2013).
 B
 A
 18
 C
 A A

Discussion

Methodological concerns

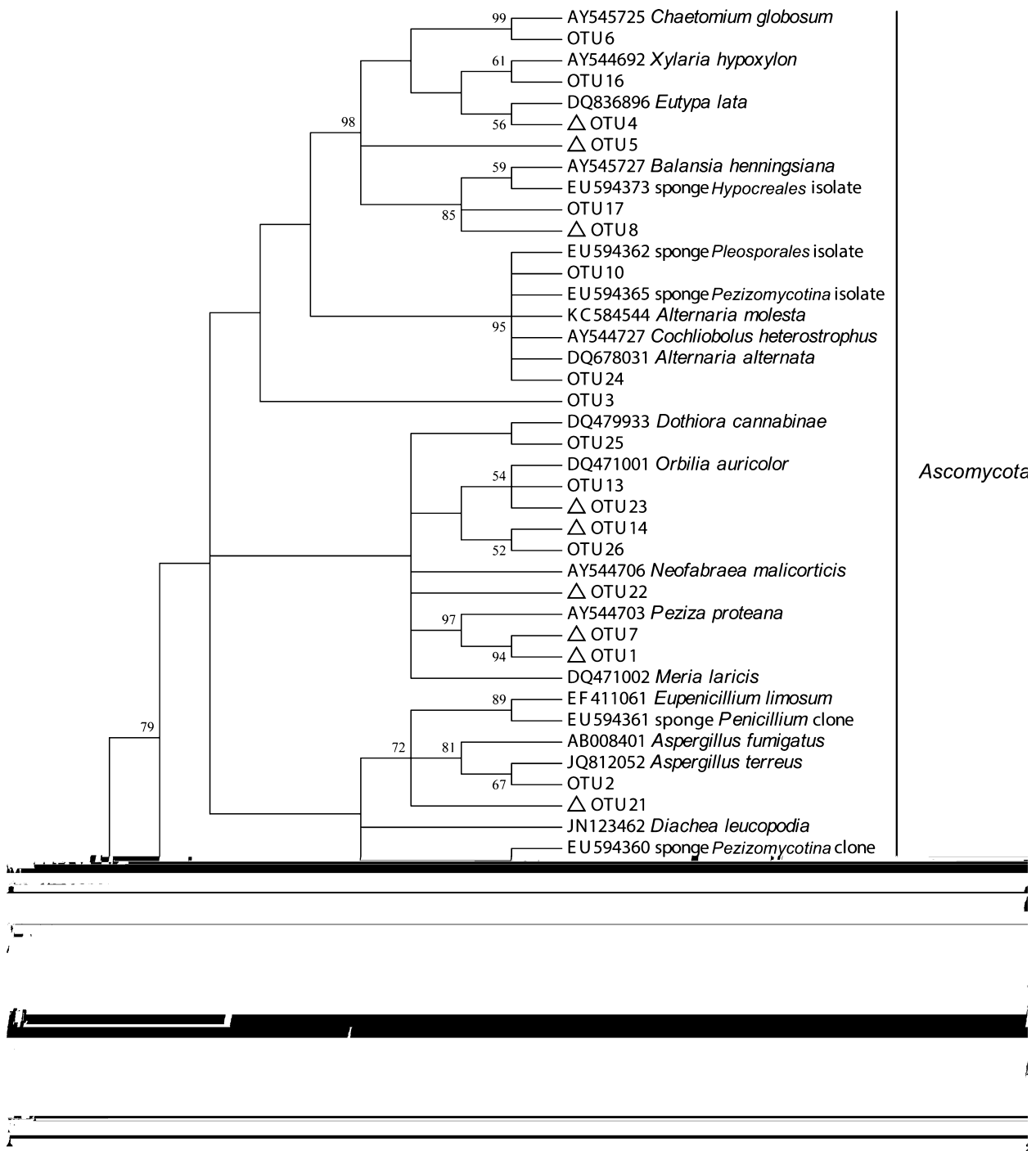


Fig. 2. Phylogenetic tree based on V2–V3 region of fungal 18S rRNA gene sequences (c. 550 bp) built by maximum-likelihood method using K2 + G as model; maximum parsimony tree and neighbor-joining tree (Supporting Information, Fig. S1) provided similar topology structure. Bootstrap values (100 replicates) higher than 50% were shown. OTUs that were only found in seawater were marked with triangles. *Zoophagus insidians* was chosen as the outgroup.

l., 2007; ... l., 2012).

100% ... A. ... II ... (... 3).

A (F . 2), 18
 B A
 8, 18, 2,

Dominance of Pezizomycotina in *T. swinhoei* and *X. testudinaria*

A.
 (l., 2006). 75%
 (3),
 (2 10) D A
 A D A
 l., 2009). 2,
 (3),
 10
 D A (6.8% . 25.5%). B
 6, 11, 16 –
 D A
 A
 C (D l., 2011; l., 2013).
 (B. l., 2009).

Comparison of fungal diversity between sponge holobionts and seawater

, D E
 (l., 2008).
 (F . 1). A A
 A (l., 2013).
 D A (2)
 , . . 8, 21,
 (3),

(l., 2010).
 l. (2012)
 D
 . B
 l. (2008) B. l. (2009),
 , 21 A. (B l l , B l l , l ,
 C l , C l , Cl l , D l ,
 l , D l , E l , l l , l ,
 l , l l , l , l , ll -
 l , l l , l l , l , -
 l , l l , l l)
 B (A l , A l l , C l ,
 l l , l l , ll , ll l ,
 ll l) (l ,
 2000; B l , 2005; , 2006; l , 2008;
 l , 2008, 2010; l , 2008; B l ,
 2009; & , 2009; l , 2010; l , 2010;
 D l , 2011; l , 2011; l , 2011;
 l , 2011; , 2012;
 l , 2012; l , 2013),
 (l l l)
 ,
 (l , 2012; l ,
 2012).
 (l , 2012).
 (l , 2000; l , 2010).
 A. 70%
 (l , 2010). , C l , E l ,
 l , l l l (D A l , 1995;
 l , 2000; l , 2002; l ,
 2004).
 (D l , 2011; l , 2013),
 (. . ,)

(1988).

B, C & (2008)

74: 6091–6101.

, F AE, 921 3()-334 ,3() .1() C .3(E)

(2012).

Acknowledgements

D C (2013AA092901).
F C (FC)
(41176127).

References

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B & (2000) *Journal of Applied Ecology* **66**: 4356–4360.

C, (2010) *Ecology Letters* **7**: 335–336.

C, C, A, C, E & C. (1994) *Journal of Applied Ecology* **59**: 6344–6348.

D'A & (1995) *Journal of Applied Ecology* **58**: 121–123.

D, B, A, C & (2001) *Journal of Applied Ecology* **67**: 2932–2941.

D, B, F & (2011) *Journal of Applied Ecology* **13**: 713–721.

... *IE* 123: 1348-1363.
'B E, ... A, ... &
(2005) F ...
... *A* *IE*
... 171: 5544-5550.
& ... (2005)
... *B* ... 48:
332-347.

C, *Environ Biol Fish* (2014) A
 506: 58–62.
 B, *Environ Biol Fish* & (2013)
 C
Environ Biol Fish 58:
 127–141.
 F & (2011)
 (), ()

C
 E 1
 62: 644–654.

Supporting Information

A

Fig. S1. 2– 3
 18 A (. 550)