

#### Supplementary Material 4

A list of the 31 regions of alignment ambiguity (RAA), 6 regions of slipped-strand compensation (RSC), and 8 regions of expansion and contraction (REC) created in the multiple sequence alignment of the expansion segments D2 and D3 of the 28S LSU rRNA from 527 sampled chalcidoids.

Ambiguous region	length <sup>a</sup> (nts)	non-homologous position <sup>b</sup>	General comments
RAA (1)	0-5	202-203	- forms a lateral bulge between strands <b>2a</b> and <b>2b</b>
RAA (2)	0-2	227-228	- forms a lateral bulge between strands <b>2e</b> and <b>2f</b>
RSC (1)	0-10	235-236	- inconsistent basepairing formed with <b>RSC (1')</b>
REC (1)	0-12	241-242	- inconsistent basepairing formed with <b>REC (1')</b> ; homology assignment beyond 5th basepair of <b>2g</b> ambiguous
RAA (3)	3-6	241-242	- terminal bulge formed by hairpin-stem <b>2g</b> + <b>REC (1)</b>
REC (1')	0-11	241-242	- inconsistent basepairing formed with <b>REC (1')</b> ; homology assignment beyond 5th basepair of <b>2g</b> ambiguous
RSC (1')	1-13	248-249	- inconsistent basepairing formed with <b>RSC (1')</b>
RAA (4)	0-5	271-272	- forms a lateral bulge between strands <b>2d'</b> and <b>2c'</b>
RAA (5)	0-4	275-276	- forms a lateral bulge between strands <b>2c'</b> and <b>2b'</b>
RAA (6)	0-3	280-281	- forms a lateral bulge between strands <b>2b'</b> and <b>2a'</b>
RAA (7)	2-16	342-343	- forms a lateral bulge between strands <b>3c</b> and <b>3d</b>
RSC (2)	0-3	354-355	- inconsistent basepairing formed with <b>RSC (2')</b>

<b>RSC (3)</b>	0-6	376-377	- inconsistent basepairing formed with <b>RSC (3')</b>
<b>REC (2)</b>	0-3	382-383	- inconsistent basepairing formed with <b>REC (2')</b> ; homology assignment beyond 5th basepair of <b>3f-3</b> ambiguous
<b>RAA (8)</b>	3-7	382-383	- terminal bulge formed by hairpin-stem <b>3f-3</b> + <b>REC (2)</b>
<b>REC (2')</b>	0-3	382-383	- inconsistent basepairing formed with <b>REC (2)</b> ; homology assignment beyond 5th basepair of <b>3f-3</b> ambiguous
<b>RSC (3')</b>	0-7	388-389	- inconsistent basepairing formed with <b>RSC (3)</b>
<b>RSC (2')</b>	0-2	406-407	- inconsistent basepairing formed with <b>RSC (2)</b>
<b>RAA (9)</b>	0-11	412-413	- forms a lateral bulge between strands <b>3e'</b> and <b>3d'</b>
<b>RAA (10)</b>	0-5	415-416	- forms a lateral bulge between strands <b>3d'</b> and <b>3c'</b>
<b>RAA (11)</b>	7-23	435-436	- stretch of unpaired nucleotides linking strands <b>3a'</b> and <b>3g</b>
<b>RAA (12)</b>	1-4	441-442	- forms a lateral bulge between strands <b>3g</b> and <b>3h</b>
<b>REC (3)</b>	0-5	450-451	- inconsistent basepairing formed with <b>REC (3')</b> ; homology assignment beyond 4th basepair of <b>3i</b> ambiguous
<b>RAA (13)</b>	3-7	450-451	- terminal bulge formed by hairpin-stem <b>3i</b> + <b>REC (3)</b>
<b>REC (3')</b>	0-5	450-451	- inconsistent basepairing formed with <b>REC (3')</b> ; homology assignment beyond 4th basepair of <b>3i</b> ambiguous
<b>RAA (14)</b>	0-4	458-459	- forms a lateral bulge between strands <b>3j</b> and <b>3k-1</b>
<b>RAA (15)</b>	0-6	481-482	- forms a lateral bulge between strands <b>3m</b> and <b>3k-n</b>
<b>RSC (4)</b>	1-8	490-491	- inconsistent basepairing formed with <b>REC (4')</b> ;
<b>RAA (16)</b>	3-5	496-497	- terminal bulge formed by hairpin-stem <b>3p</b>
<b>RAA (17)</b>	1-3	498-499	- forms a lateral bulge between strands <b>3p'</b> and <b>3o'</b>

<b>RSC (4')</b>	1-2	528-529	- inconsistent basepairing formed with <b>REC (4)</b> ;
<b>RAA (18)</b>	1-11	512-513	- forms a lateral bulge between strands <b>3n'</b> and <b>3m'</b>
<b>RAA (19)</b>	0-7	515-516	- forms a lateral bulge between strands <b>3m'</b> and <b>3l'</b>
<b>RAA (20)</b>	3-9	522-523	- forms a lateral bulge between strands <b>3l'</b> and <b>3k-2'</b>
<b>RAA (21)</b>	0-2	527-528	- forms a lateral bulge between strands <b>3k-2'</b> and <b>3k-1'</b>
<b>RAA (22)</b>	0-3	544-545	- forms a lateral bulge between strands <b>3j'</b> and <b>3h'</b>
<b>RAA (23)</b>	0-9	549-550	- forms a lateral bulge between strands <b>3h'</b> and <b>REC (4)</b> , or " <b>3q</b> "
<b>REC (4)</b>	0-17	549-550	- inconsistent basepairing formed with <b>REC (4')</b> ; homology assignment within putative helix <b>3q</b> ambiguous
<b>RAA (24)</b>	3-10	549-550	- terminal bulge formed by hairpin-stem <b>REC (4)</b> , or " <b>3q</b> "
<b>REC (4')</b>	0-17	549-550	- inconsistent basepairing formed with <b>REC (4)</b> ; homology assignment within putative helix <b>3q</b> ambiguous
<b>RAA (25)</b>	0-13	549-550	- forms a lateral bulge between strands <b>REC (4)</b> , or " <b>3q</b> " and <b>3g'</b>
<b>REC (5)</b>	0-14	641-642	- inconsistent basepairing formed with <b>REC (5')</b> ; homology assignment beyond 9th basepair of <b>H604</b> ambiguous
<b>RAA (26)</b>	3-16	641-642	- terminal bulge formed by hairpin-stem <b>H604</b>
<b>REC (5')</b>	0-14	641-642	- inconsistent basepairing formed with <b>REC (5)</b> ; homology assignment beyond 9th basepair of <b>H604</b> ambiguous
<b>RAA (27)</b>	0-2	670-671	- forms a lateral bulge between strands <b>D3-1a</b> and <b>D3-1b</b>
<b>RAA (28)</b>	0-3	671-672	- forms a lateral bulge between strands <b>D3-1b</b> and <b>D3-1c</b>
<b>REC (6)</b>	0-18	675-676	- inconsistent basepairing formed with <b>REC (6')</b> ; homology assignment beyond 3rd basepair of <b>D3-1c</b> ambiguous

<b>RAA (29)</b>	3-8	675-676	- terminal bulge formed by hairpin-stem <b>D3-1c</b>
<b>REC (6')</b>	0-18	675-676	- inconsistent basepairing formed with <b>REC (6)</b> ; homology assignment beyond 3rd basepair of <b>D3-1c</b> ambiguous
<b>RSC (5)</b>	0-6	702-703	- inconsistent basepairing formed with <b>RSC (5')</b> ;
<b>REC (7)</b>	0-10	702-703	- inconsistent basepairing formed with <b>REC (7')</b> ; homology assignment beyond 3rd basepair of <b>D3-2b</b> ambiguous
<b>RAA (30)</b>	3-7	702-703	- terminal bulge formed by hairpin-stem <b>D3-2b</b>
<b>REC (7')</b>	0-10	702-703	- inconsistent basepairing formed with <b>REC (7)</b> ; homology assignment beyond 3rd basepair of <b>D3-2b</b> ambiguous
<b>RSC (5')</b>	0-4	705-706	- inconsistent basepairing formed with <b>RSC (5)</b> ;
<b>RSC (6)</b>	2-3	735-736	- inconsistent basepairing formed with <b>RSC (6')</b> ;
<b>REC (8)</b>	0-10	739-740	- inconsistent basepairing formed with <b>REC (8')</b> ; homology assignment beyond 4th basepair of <b>D3-3b</b> ambiguous
<b>RAA (31)</b>	4-6	739-740	- terminal bulge formed by hairpin-stem <b>D3-3b</b>
<b>REC (8')</b>	0-10	739-740	- inconsistent basepairing formed with <b>REC (8)</b> ; homology assignment beyond 4th basepair of <b>D3-3b</b> ambiguous
<b>RSC (6')</b>	1-1	743-744	- inconsistent basepairing formed with <b>RSC (6)</b>

<sup>a</sup> Refers to the range of nucleotides within each ambiguous region.

<sup>b</sup> Nucleotide positions flanking ambiguous regions are given in Figure 2.