

TAXON

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Electronic Supplement to

**Phylogenetic study of *Diploschistes*
(lichen-forming Ascomycota: Ostropales:
Graphidaceae), based on morphological, chemical,
and molecular data**

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***Taxon* 62: 267–280**

Table S1. List of character states for one ecological (1), 24 morphological (2–25), and seven chemical (26–32) characters selected for phylogenetic analyses of 56 taxa. Characters excluded for being uninformative are marked with an asterisk. Numbers in parentheses following color names (character 3) correspond to the color code by Kornerup & Wanscher (1981, *Taschenlexikon der Farben*, 3rd ed. Göttingen: Muster-Schmidt Verlag).

Character name	Character states	Character name	Character states
1. Substrate type	0: siliceous rock 1: carbonate rock 2: soil 3: lichen/moss 4: bark	16. Ascus length	0: short ($\leq 100 \mu\text{m}$) 1: medium ($> 100\text{--}125 \mu\text{m}$) 2: long ($> 125 \mu\text{m}$)
2. Photobiont type	0: trebouxoid 1: trentepohlioid	17. Ascus width	0: narrow ($13\text{--}23 \mu\text{m}$) 1: wide ($> 23 \mu\text{m}$)
3. Thallus color	0: white (-A1) 1: grayish white (-B1) 2: grayish yellow (2B3) 3: orange gray (5B2) 4: bluish white (22A2, 23A2) 5: bluish gray (23B2, 23D3) 6: greenish white (25A2, 26A2) 7: greenish gray (25B2, 28B2) 8: grayish brown (6D3)	18. Ascospores per ascus	0: 4 spores per ascus 1: 6–8 spores per ascus
4. Pruina on thallus upper surface	0: absent 1: present	19. Ascospore length	0: very short ($\leq 25 \mu\text{m}$) 1: short ($> 25\text{--}29 \mu\text{m}$) 2: long ($> 29\text{--}33 \mu\text{m}$) 3: very long ($> 33 \mu\text{m}$)
5. Thallus type	0: continuous-rimose 1: rimose-areolate 2: verrucose-areolate	20. Ascospore width	0: very narrow ($\leq 10 \mu\text{m}$) 1: narrow ($> 10\text{--}16 \mu\text{m}$) 2: wide ($> 16\text{--}20 \mu\text{m}$) 3: very wide ($> 20 \mu\text{m}$)
6. Thallus thickness	0: thin ($0.2\text{--}0.8 \text{ mm}$) 1: thick ($> 0.8 \text{ mm}$)	21. Ascospore shape (Length/Width ratio)	0: broadly ellipsoid (≤ 2.0) 1: ellipsoid ($> 2.0\text{--}2.4$) 2: narrow ellipsoid ($> 2.4\text{--}2.8$) 3: very narrow (> 2.8)
7. Thallus cortical layer type	0: absent 1: cortex 2: protocortex	22. Number of transverse septa	0: ≤ 5 septa 1: 6–8 septa 2: > 8 septa
8. Thallus epinecral layer thickness	0: thin ($10\text{--}40 \mu\text{m}$) 1: thick ($> 40 \mu\text{m}$)	23. Number of longitudinal septa	0: absent 1: mainly 1 septum 2: mainly 2 or more septa
* Algal layer thickness	μm	24. Number of locules per ascospore	0: ≤ 12 locules 1: $> 13\text{--}17$ locules 2: 18–22 locules 3: ≥ 23 locules
9. Ascoma presence	0: absent 1: present	25. I reaction of the spores	0: I– 1: I+
10. Ascoma type	0: perithecioid 1: urceolate 2: lecanoroid 3: lepadoid	26. K reaction of the thallus	0: K– 1: K+ yellow 2: K+ yellow turning red
11. Ascoma diameter	0: small ($\leq 0.8 \text{ mm}$) 1: medium ($> 0.8\text{--}1.5 \text{ mm}$) 2: large ($> 1.5 \text{ mm}$)	27. C reaction of the thallus	0: C– 1: C+ red
12. Excipulum development	0: reduced 1: well-developed	28. I reaction of the medulla	0: I– 1: I+ blue or purple
13. Excipulum color	0: colorless to pale brown 1: dark brown to black	29. Lecanoric acid	0: not detected 1: detected
* Excipulum thickness	μm	30. Diploschistesic acid	0: not detected 1: detected
14. Lateral paraphyses	0: absent 1: present	31. Orsellinic acid	0: not detected 1: detected
15. Hymenium thickness	0: thin ($85\text{--}130 \mu\text{m}$) 1: thick ($> 130 \mu\text{m}$)	32. Stictic acid aggregates	0: not detected 1: detected

Table S2. Data matrix for ecological (1), morphological (2–25), and chemical (26–32) characters. Continuous characters for specimens within each taxon were scored identically. Characters and character states are described in Table S1. “?” = character state unknown. For certain characters, some specimens were assigned multiple character states because they were polymorphic and were analyzed as such: A = 0&1; B = 1&2; C = 2&7. Polymorphisms were analyzed using the Variable option in PAUP*.

	1. Substrate type	2. Photobiont type	3. Thallus color	4. Pruina on thallus upper surface	5. Thallus type	6. Thallus thickness	7. Thallus cortical layer type	8. Thallus epinecral layer thickness	9. Ascoma presence	10. Ascoma type	11. Ascoma diameter	12. Excipulum development	13. Excipulum color	14. Lateral paraphyses	15. Hymenium thickness	16. Ascus length	17. Ascus width	18. Ascospores per ascus	19. Ascospore length	20. Ascospore width	21. Ascospore shape (length/width ratio)	22. Number of transverse septa	23. Number of longitudinal septa	24. Number of locules per ascospore	25. I reaction of the spores	26. K reaction of the thallus	27. C reaction of the thallus	28. I reaction of the medulla	29. Lecanoric acid	30. Diploshistic acid	31. Orsellinic acid	32. Stictic acid aggregates
<i>D. actinostomus</i> 1	0	0	3	0	0	A	0	0	1	0	0	1	1	1	1	1	A	1	B	B	A	A	2	B	0	0	1	1	1	1	0	0
<i>D. actinostomus</i> 2	0	0	3	0	0	A	0	0	1	0	0	1	1	1	1	1	A	1	B	B	A	A	2	B	0	0	1	1	1	1	0	0
<i>D. caesioplumbeus</i> 1	0	0	5	0	0	A	0	0	1	0	0	1	1	1	1	B	A	1	2	2	A	1	2	2	0	0	1	1	1	0	0	0
<i>D. caesioplumbeus</i> 2	0	0	5	0	0	A	0	0	1	0	0	1	1	1	1	B	A	1	2	2	A	1	2	2	0	0	1	1	1	0	0	0
<i>D. caesioplumbeus</i> 3	0	0	5	0	0	A	0	0	1	0	0	1	1	1	1	B	A	1	2	2	A	1	2	2	0	0	1	1	1	0	0	0
<i>D. candidissimus</i> 1	1	0	4	1	0	A	0	0	1	0	0	1	1	1	1	2	0	1	2	2	A	1	2	2	0	0	1	1	1	0	0	0
<i>D. candidissimus</i> 2	1	0	4	1	0	A	0	0	1	0	0	1	1	1	1	2	0	1	2	2	A	1	2	2	0	0	1	1	1	0	0	0
<i>D. diacapsis</i> 1	2	0	A	1	1	A	0	A	1	1	A	1	1	1	0	1	0	1	1	1	A	0	1	0	0	1	1	0	?	?	?	?
<i>D. diacapsis</i> 2	2	0	A	1	1	A	0	A	1	1	A	1	1	1	0	1	0	1	1	1	A	0	1	0	0	1	1	0	?	?	?	?
<i>D. diacapsis</i> 3	2	0	A	1	1	A	0	A	1	1	A	1	1	1	0	1	0	1	1	1	A	0	1	0	0	1	1	0	1	1	0	0
<i>D. diacapsis</i> 4	2	0	0	1	1	A	0	A	1	1	A	1	1	1	0	1	0	1	1	1	A	0	1	0	0	1	1	0	1	1	0	0
<i>D. diacapsis</i> 5	2	0	0	1	1	A	0	A	1	1	A	1	1	1	0	1	0	1	1	1	A	0	1	0	0	1	1	0	1	0	0	0
<i>D. diacapsis</i> 6	2	0	0	1	1	A	0	A	1	1	A	1	1	1	0	1	0	1	1	1	A	0	1	0	0	1	1	0	1	1	0	0
<i>D. diploschistoides</i> 1	0	0	1	0	0	0	0	0	1	0	0	1	1	1	0	A	A	1	1	B	A	0	B	1	1	0	1	1	1	0	0	0
<i>D. diploschistoides</i> 2	0	0	5	0	0	0	0	0	1	0	0	1	1	1	0	A	A	1	1	B	A	0	B	1	1	0	1	1	1	0	0	0
<i>D. euganeus</i>	0	0	8	0	0	0	0	0	1	0	0	1	1	1	A	1	0	1	1	2	0	0	2	B	0	0	0	0	0	0	0	0
<i>D. gypsaceus</i> 1	1	0	4	1	1	1	0	0	1	1	A	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	0	0	0
<i>D. gypsaceus</i> 2	1	0	6	1	1	1	0	0	1	1	A	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	0	0	0
<i>D. gypsaceus</i> 3	1	0	7	1	1	1	0	0	1	1	A	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	0	0	0
<i>D. gypsaceus</i> 4	1	0	4	1	1	1	0	0	1	1	A	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	?	?	?	?
<i>D. gypsaceus</i> 5	1	0	7	1	1	1	0	0	1	1	A	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	0	0	0
<i>D. interpediens</i> 1	0	0	2	0	1	A	0	0	1	1	1	1	1	1	0	A	0	1	1	1	1	0	B	A	0	1	1	0	1	1	1	0
<i>D. interpediens</i> 2	0	0	6	0	1	A	0	0	1	1	1	1	1	1	0	A	0	1	1	1	1	0	B	A	0	1	1	0	1	1	1	0
<i>D. interpediens</i> 3	0	0	2	0	1	A	0	0	1	1	1	1	1	1	0	A	0	1	1	1	1	0	B	A	0	1	1	0	1	1	0	0
<i>D. interpediens</i> 4	0	0	6	0	1	A	0	0	1	1	1	1	1	1	0	A	0	1	1	1	1	0	B	A	0	1	1	0	1	1	1	0
<i>D. interpediens</i> 5	0	0	2	0	1	A	0	0	1	1	1	1	1	1	0	A	0	1	1	1	1	0	B	A	0	1	1	1	1	1	1	0
<i>D. interpediens</i> 6	0	0	7	0	1	A	0	0	1	1	1	1	1	1	0	A	0	1	1	1	1	0	B	A	0	1	1	0	1	1	0	0
<i>D. interpediens</i> 7	0	0	2	0	1	A	0	0	1	1	1	1	1	1	0	A	0	1	1	1	1	0	B	A	0	1	1	0	1	1	1	0
<i>D. interpediens</i> 8	0	0	7	0	1	A	0	0	1	1	1	1	1	1	0	A	0	1	1	1	1	0	B	A	0	1	1	1	1	1	1	0
<i>D. interpediens</i> 9	0	0	2	0	1	A	0	0	1	1	1	1	1	1	0	A	0	1	1	1	1	0	B	A	0	1	1	0	1	1	1	0
<i>D. interpediens</i> 10	0	0	7	0	1	A	0	0	1	1	1	1	1	1	0	A	0	1	1	1	1	0	B	A	0	1	1	1	1	1	1	0
<i>D. muscorum</i> 1	3	0	7	0	1	0	0	0	1	1	A	1	1	1	0	0	0	0	1	1	1	0	1	0	0	1	1	0	1	1	1	0
<i>D. muscorum</i> 2	3	0	7	0	1	0	0	0	1	1	A	1	1	1	0	0	0	0	1	1	1	0	1	0	0	1	1	0	1	1	1	0
<i>D. muscorum</i> 3	3	0	7	0	1	0	0	0	1	1	A	1	1	1	0	0	0	0	1	1	1	0	1	0	0	1	1	0	1	1	1	0

Table S2. Continued.

	1. Substrate type	2. Photobiont type	3. Thallus color	4. Pruina on thallus upper surface	5. Thallus type	6. Thallus thickness	7. Thallus cortical layer type	8. Thallus epinecral layer thickness	9. Ascoma presence	10. Ascoma type	11. Ascoma diameter	12. Excipulum development	13. Excipulum color	14. Lateral paraphyses	15. Hymenium thickness	16. Ascus length	17. Ascus width	18. Ascospores per ascus	19. Ascospore length	20. Ascospore width	21. Ascospore shape (length/width ratio)	22. Number of transverse septa	23. Number of longitudinal septa	24. Number of locules per ascospore	25. I reaction of the spores	26. K reaction of the thallus	27. C reaction of the thallus	28. I reaction of the medulla	29. Lecanoric acid	30. Diploschistic acid	31. Orsellinic acid	32. Stictic acid aggregates
<i>D. muscorum</i> 4	3	0	7	0	1	0	0	0	1	1	A	1	1	1	0	0	0	0	1	1	1	0	1	0	0	1	1	0	1	1	1	0
<i>D. muscorum</i> 5	3	0	7	0	1	0	0	0	1	1	A	1	1	1	0	0	0	0	1	1	1	0	1	0	0	1	1	0	?	?	?	?
<i>D. ocellatus</i> 1	1	0	0	1	2	1	1	1	2	2	0	0	0	1	1	0	1	0	0	B	0	1	0	0	2	0	0	0	0	0	0	1
<i>D. ocellatus</i> 2	2	0	0	1	2	1	1	1	2	2	0	0	0	1	1	0	1	0	0	B	0	1	0	0	2	0	0	0	0	0	0	1
<i>D. ocellatus</i> 3	1	0	0	1	2	1	1	1	0	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	2	0	0	0	0	0	0	1
<i>D. ocellatus</i> 4	1	0	0	1	2	1	1	1	0	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	2	0	0	0	0	0	0	1
<i>D. ocellatus</i> 5	1	0	0	1	2	1	1	1	2	2	0	0	0	1	1	0	1	0	0	B	0	1	0	0	2	0	0	0	0	0	0	1
<i>D. rampoddensis</i> 1	0	0	2	0	1	A	0	0	1	1	A	1	1	1	0	A	0	1	A	A	1	1	1	A	0	1	1	0	1	0	0	0
<i>D. rampoddensis</i> 2	0	0	2	0	1	A	0	0	1	1	A	1	1	1	0	A	0	1	A	A	1	1	1	A	0	1	1	0	1	0	0	0
<i>D. scruposus</i> 1	0	0	2	0	1	A	0	0	1	1	1	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	1	1	0	0	0
<i>D. scruposus</i> 2	0	0	6	0	1	A	0	0	1	1	1	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	1	1	0
<i>D. scruposus</i> 3	0	0	2	0	1	A	0	0	1	1	1	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	1	1	1	0	0
<i>D. scruposus</i> 4	0	0	6	0	1	A	0	0	1	1	1	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	1	1	0
<i>D. scruposus</i> 5	0	0	6	0	1	A	0	0	1	1	1	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	0	1	0
<i>D. scruposus</i> 6	0	0	6	0	1	A	0	0	1	1	1	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	1	1	0
<i>D. scruposus</i> 7	0	0	7	0	1	A	0	0	1	1	1	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	1	1	0
<i>D. scruposus</i> 8	0	0	6	0	1	A	0	0	1	1	1	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	1	1	0
<i>D. scruposus</i> 9	0	0	2	0	1	A	0	0	1	1	1	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	1	0	0
<i>D. scruposus</i> 10	0	0	6	0	1	A	0	0	1	1	1	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	0	1	1	1	0
<i>D. scruposus</i> 11	0	0	2	0	1	A	0	0	1	1	1	1	1	1	0	A	0	0	B	1	1	1	B	1	0	1	1	1	1	1	0	0
<i>T. lepadinum</i> 1	4	1	C	0	A	0	B	0	1	3	B	1	0	1	1	?	?	A	3	3	3	2	B	3	A	0	0	?	0	0	0	0
<i>T. suecicum</i> 1	4	1	C	0	A	0	B	0	1	3	A	1	0	1	A	?	?	1	2	1	2	1	0	0	A	0	0	?	0	0	0	0

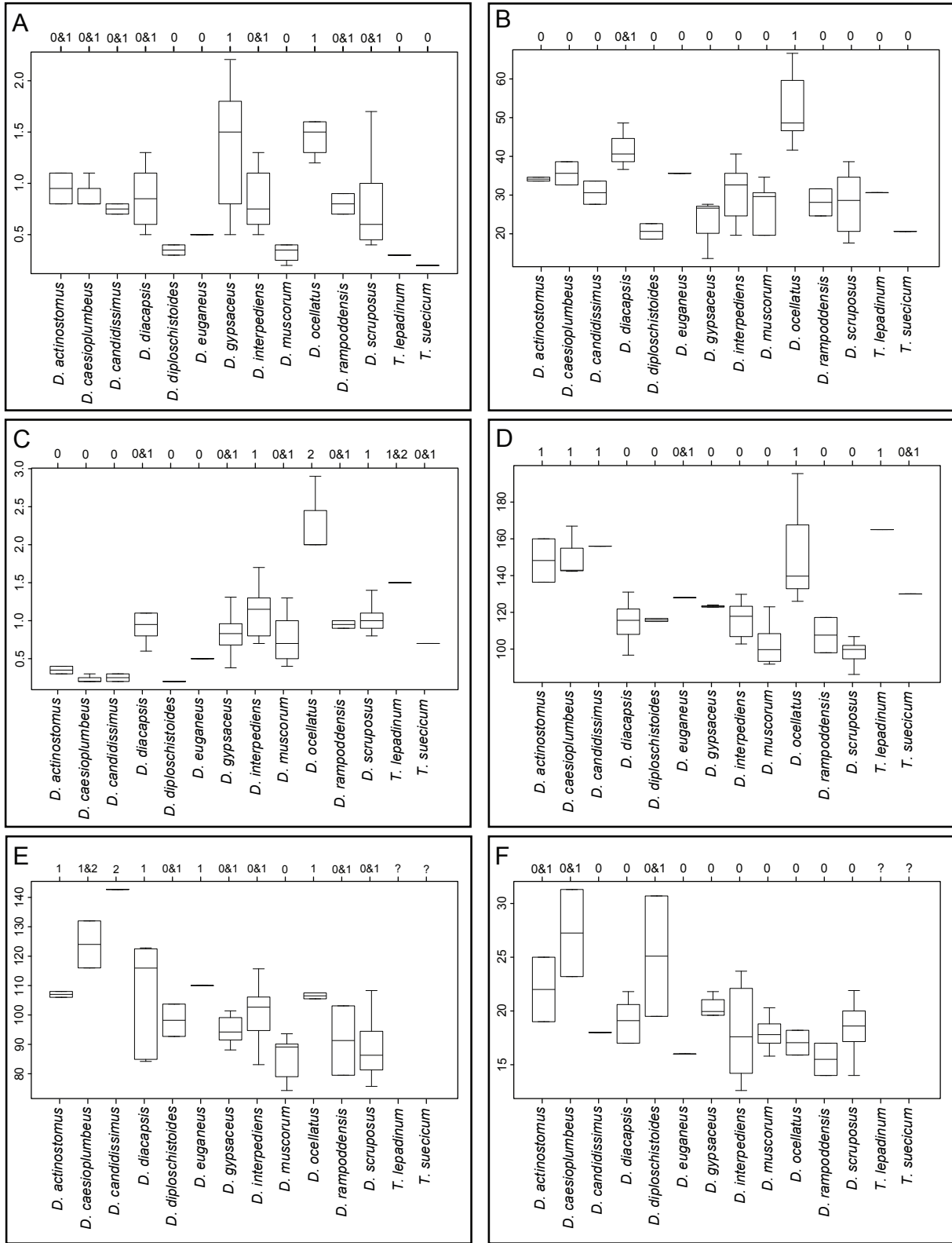


Fig. S1 (part 1/2). Conversion of continuous characters to discrete. Box plots representing twelve continuous characters included in the morphological-chemical data matrix for twelve species of *Diploschistes* and two outgroup taxa. The central line within the box is the median, the boxed areas represent 50% of the sample and the vertical lines represent the whole sample. Species whose distribution for a given character did not differ significantly according to the Tukey's HSD test were given multiple characters states, shown in the upper side of each plot. **A**, thallus thickness (Table S1; char. 6); **B**, Epinecral layer thickness (Table S1; char. 8); **C**, ascoma diameter (Table S1; char. 11); **D**, hymenium thickness (Table S1; char. 15); **E**, ascus length (Table S1; char. 16); **F**, ascus width (Table S1; char. 17) ... [continued next page]

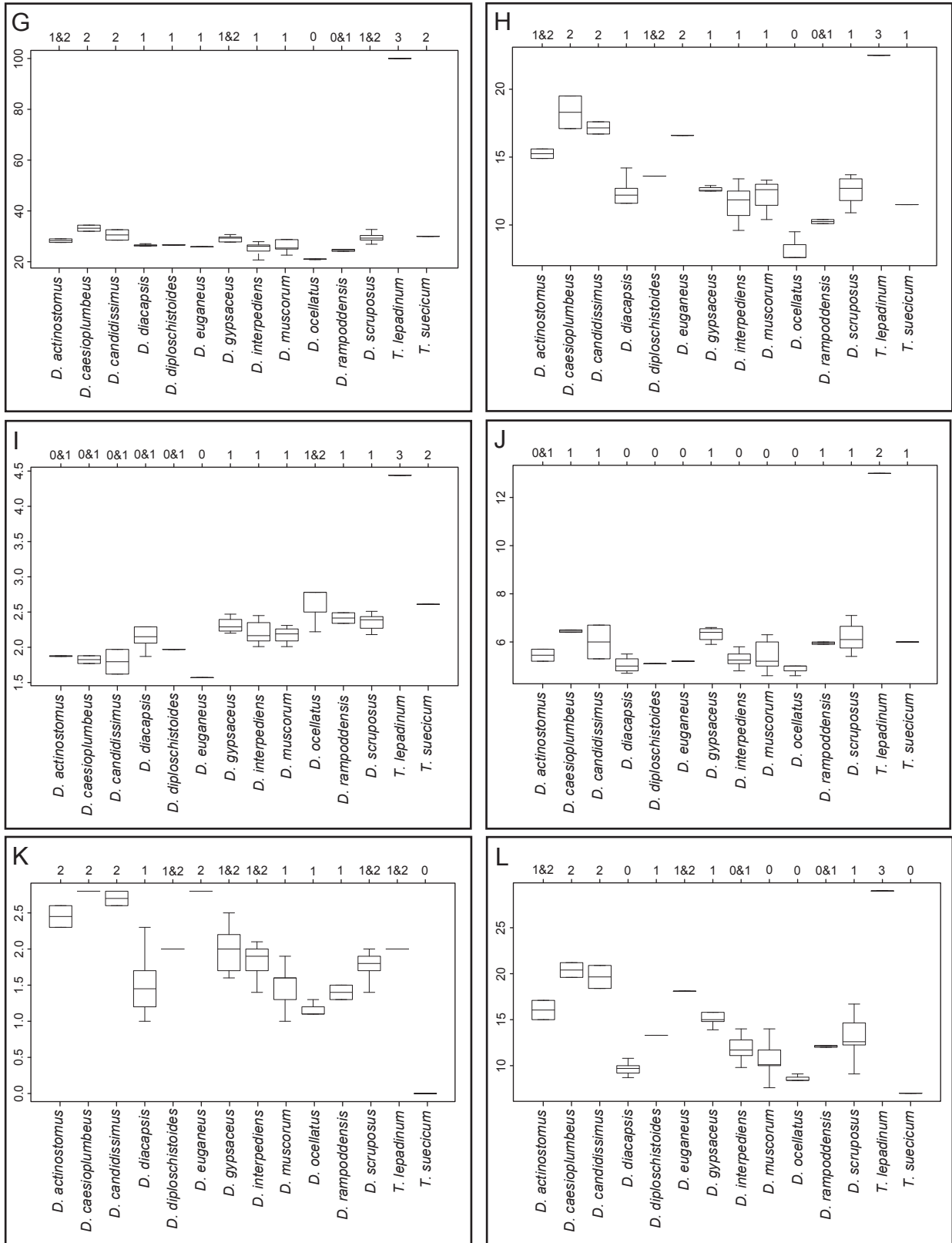


Fig. S1 (part 2/2). [continued from previous page] ... **G**, ascospore length (Table S1; char. 19); **H**, ascospore width (Table S1; char. 20); **I**, ascospore shape (length/width ratio) (Table S1; char. 21); **J**, number of transverse septa (Table 1; char. 22); **K**, number of longitudinal septa (Table S1; char. 23); **L**, number of locules per ascospore (Table S1; char. 24).

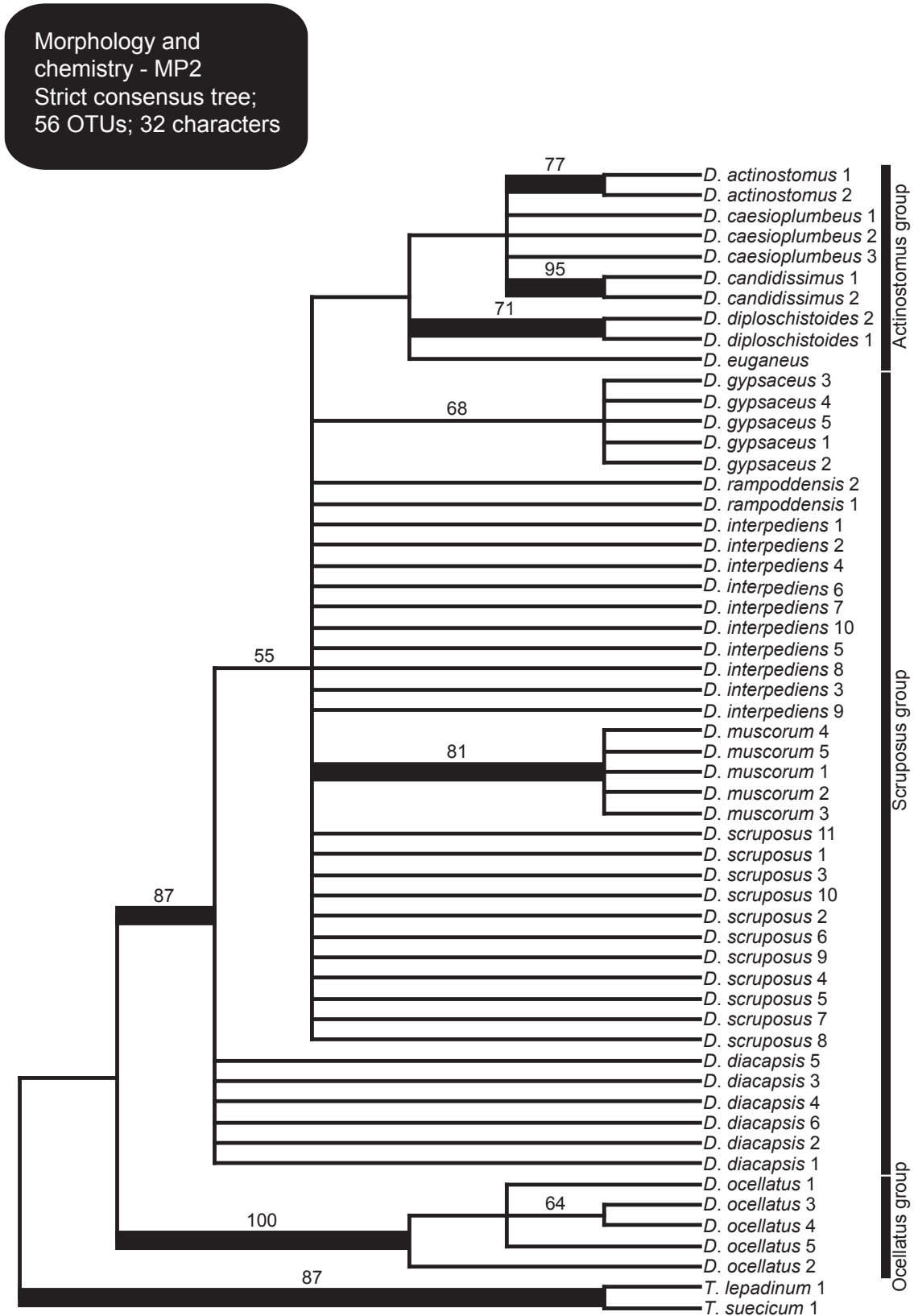


Fig. S2. Phylogenetic relationships among 54 individuals of *Diploschistes* and two outgroup species, using maximum parsimony as the optimization criterion. Strict consensus tree of 58,700 equally most parsimonious trees based on morphological-chemical data alone (MP2). Parsimony bootstrap percentages (BS) greater than 50% are shown above branches. Highly supported internodes (MPBS \geq 70%) are indicated by bold lines.

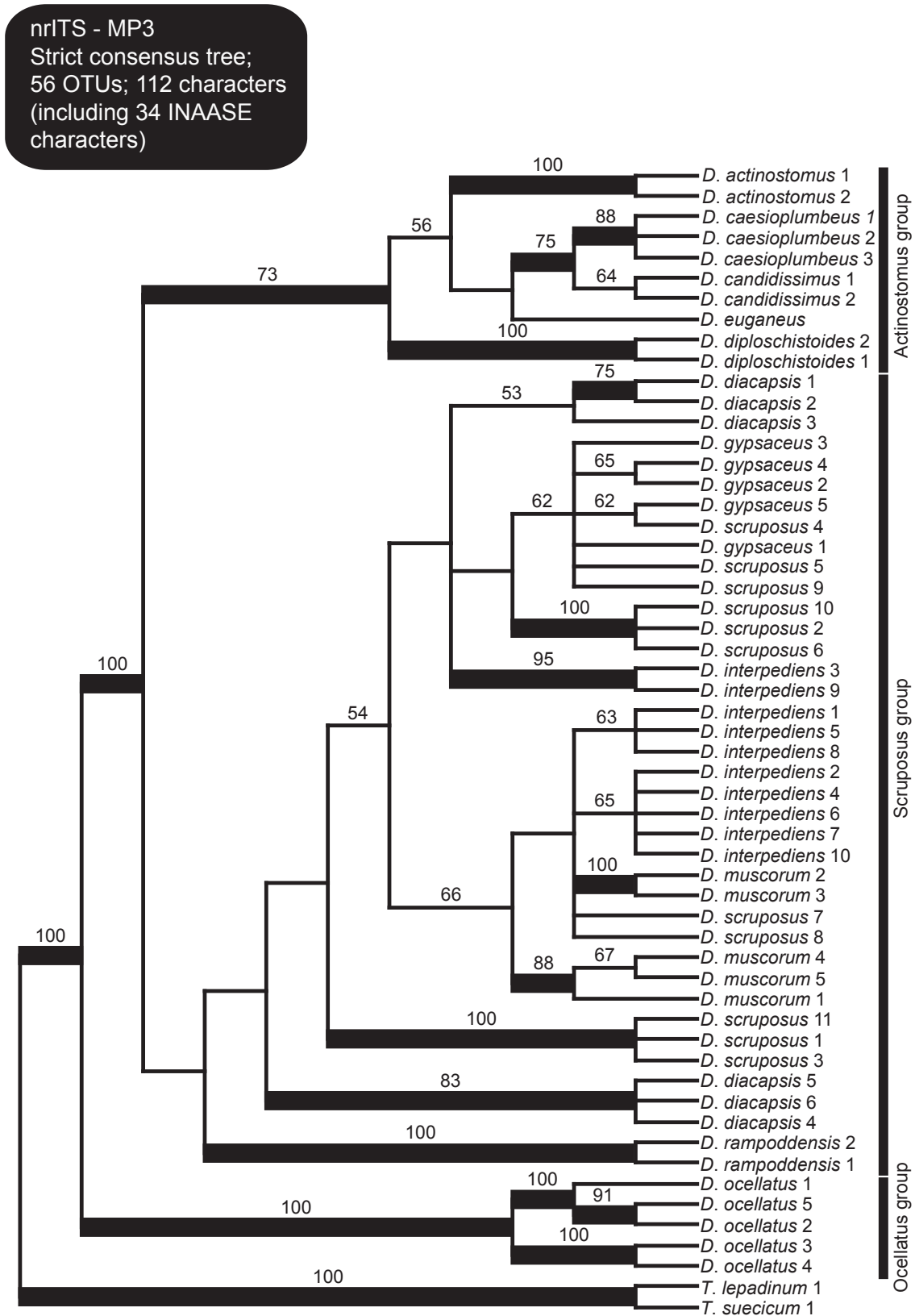


Fig. S3. Phylogenetic relationships among 54 individuals of *Diploschistes* and two outgroup species, using maximum parsimony as the optimization criterion. Strict consensus tree of six equally most parsimonious trees based on nrITS alone (MP3). Parsimony bootstrap percentages (BS) greater than 50% are shown above branches. Highly supported internodes (MPBS $\geq 70\%$) are indicated by bold lines.

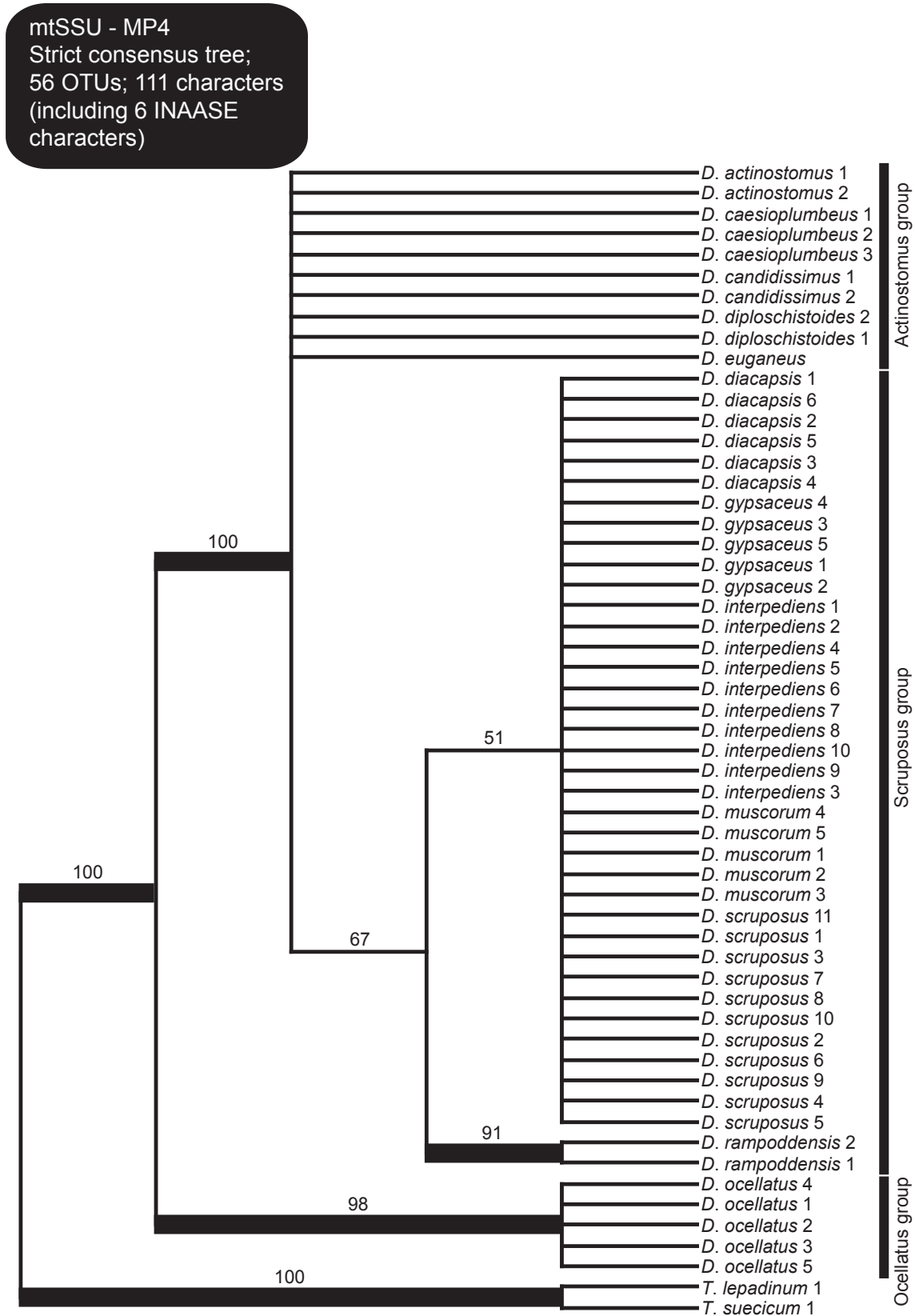


Fig. S4. Phylogenetic relationships among 54 individuals of *Diploschistes* and two outgroup species, using maximum parsimony as the optimization criterion. Strict consensus tree of 90,400 equally most parsimonious trees based on mtSSU alone (MP4). Parsimony bootstrap percentages (BS) greater than 50% are shown above branches. Highly supported internodes (MPBS \geq 70%) are indicated by bold lines.

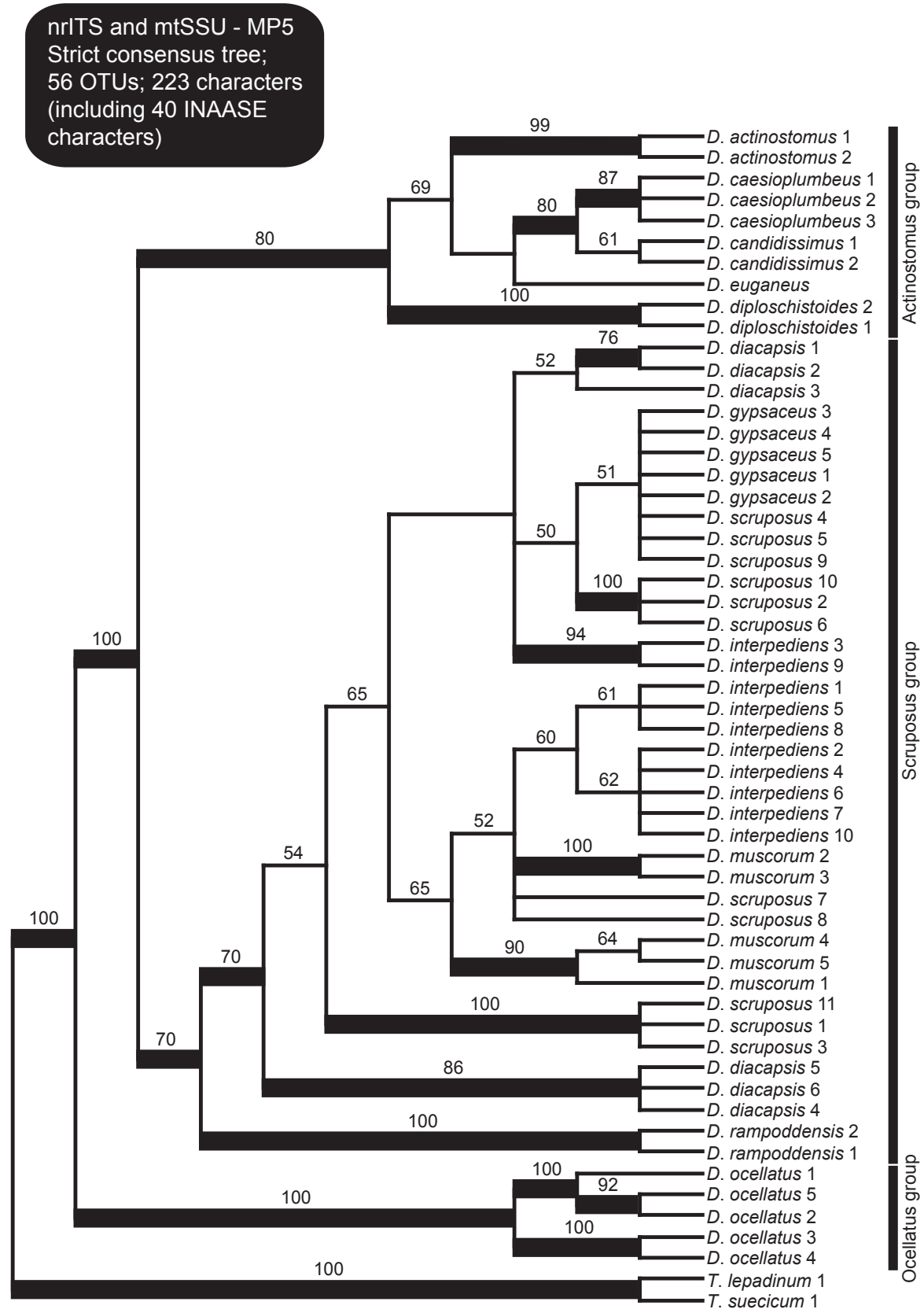


Fig. S5. Phylogenetic relationships among 54 individuals of *Diploschistes* and two outgroup species, using maximum parsimony as the optimization criterion. Strict consensus tree of 336 equally most parsimonious trees based on the nrITS and mtSSU combined dataset (MP5). Parsimony bootstrap percentages (BS) greater than 50% are shown above branches. Highly supported internodes (MPBS $\geq 70\%$) are indicated by bold lines.

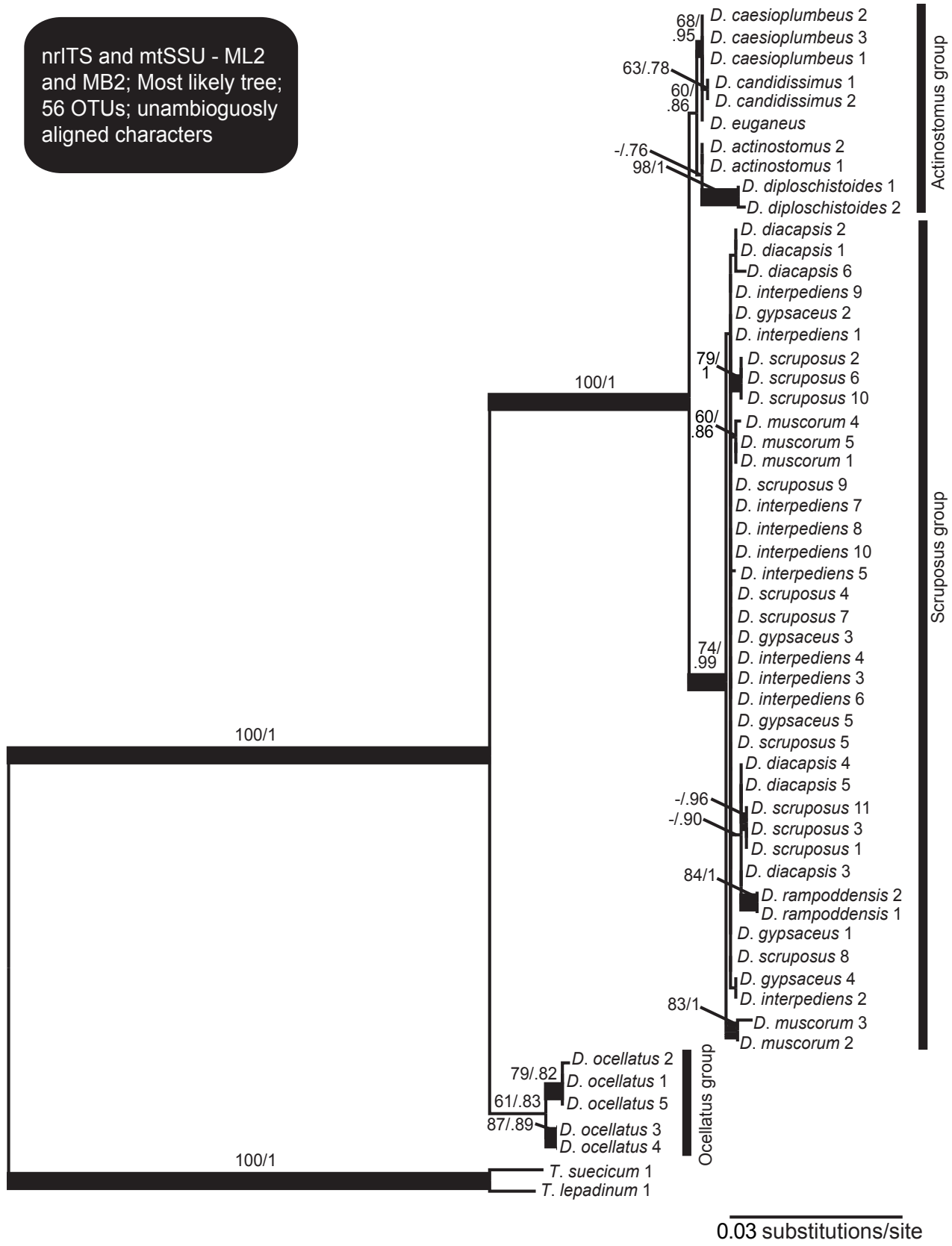


Fig. S6. Phylogenetic relationships among 54 individuals of *Diploschistes* and two outgroup species as revealed by maximum likelihood analyses of the nrITS and mtSSU combined dataset. Support values above branches indicate maximum likelihood support values and posterior probabilities, ordered as ML2BS/MB2PP. Thicker internodes indicate significant support for at least one statistical method (MLBS $\geq 70\%$, PP ≥ 0.95).