

Supplement 1: Sample collection sites.

Sample sites	Geographical coordinates
Araras – SP	22°18'41"S, 47°23'05"W
São Carlos – SP	21°59'03"S, 47°52'55"W
Rifaina – SP	20°03'32"S, 47°26'25"W
São José dos Campos – SP	23°11'01"S, 45°55'39"W
Lavras – MG	21°13'56"S, 44°59'37"W
Viçosa – MG	20°45'39"S, 42°51'50"W
Ilhéus – BA	14°47'46"S, 39°10'22"W
Campo Formoso – BA	10°21'37"S, 40°26'09"W
Guarapuava – PR	25°23'36"S, 51°27'19"W
Prudentópolis – PR	25°19'08"S, 51°01'56"W
Atílio Vivácqua – ES	20°56'32"S, 41°11'51"W
Aracajú – SE	10°54'03"S, 37°04'29"W
Ilha do Cardoso – SP	25°03'05"S, 48°05'42" W
Cananéia – SP	25°01'28"S, 47°55'56"W
Ilha Comprida – SP	24°43'53"S, 47°33'32"W
Itirapina – SP	22°15'10"S, 47°49'22"W
Cotriguaçu – MT	09°57'27"S, 58°24'49"W

Supplement 2: Information on primer sequences. T_m indicates the melting temperature in Celsius degrees.

Gene	Primers sequences	T _m	Reference
<i>Polfor2a</i>	5' - AAYAARCCVGTATGGGTATTGTRCA - 3'	54	Danforth et al., 2006
<i>Polrev2a</i>	5' - AGRTANGARTTCTCRACGAATCCTCT - 3'		
<i>Lep F1</i>	5'- ATTCAACCAATCATAAAGATATTGG - 3'	48	Hebert et al., 2003
<i>Lep R1</i>	5' - TAAACTTCTGGATGTCCAAAAAATCA- 3'		
<i>TyCytBF</i>	5' - TAAAGAGTCCAATTACTCCTCC - 3'	54	Present study
<i>TyCytBR</i>	5' - GGACAAATATCATTTTGGAGGAGC - 3'		
<i>Wg578F</i>	5' - TGCACNGTGAARACYTGCTGGATGCG - 3'	48	Ward & Downie, 2005
<i>Wg1032R</i>	5' - ACYTGCAGCACCARTGGAA - 3'		
<i>Rho F</i>	5' - TATGCAATGTTGGGCTCCTT - 3'	48-54	Present study
<i>Rho R</i>	5' - GGCAACGTTCAATTTTTTCG - 3'		
<i>EF F</i>	5' - AGTATTAATTGTTGCTGGC - 3'	48-54	Present study
<i>EF R</i>	5' - GTGGAAACCGCGAATGAG - 3'		

Supplement 3: *Trypoxylon* samples analysed for *COI* gene. Indications of subgenus, name given for the morphotype, number of sequences (N), sequence designation, and sequence identification number in the NCBI / GenBank databases.

Subgenus	Morphotype	N	Sequence	NCBI/GenBank
<i>Trypargilum</i>	Sp02	1	Nit1H2	MK413168
<i>Trypargilum</i>	Sp04	2	Sp6H1	MK413144
<i>Trypargilum</i>	<i>T. sp2</i>	1	Sp2H1	MK413130
<i>Trypargilum</i>	<i>T. sp4</i>	1	Sp4H1	MK413131
<i>Trypargilum</i>	<i>T. sp5</i>	1	Sp5H1	MK413132
<i>Trypargilum</i>	Morphotype A	4	"A"H1	MK413103 - 105
			"A"H2	MK413106
<i>Trypargilum</i>	Morphotype B	2	"B"H1	MK413107 - 108
<i>Trypargilum</i>	Morphotype C	8	"C"H1	MK413109 - 114
			"J"H1	MK413115 - 116
<i>Trypargilum</i>	Morphotype D	2	"D"H1	MK413117
			"D"H2	MK413118
<i>Trypoxylon</i>	Morphotype E	1	"E"H1	MK413120
<i>Trypargilum</i>	Morphotype F	1	"D"H1	MK413119
<i>Trypoxylon</i>	Morphotype H	2	"H"H1	MK413121 - 122
<i>Trypargilum</i>	Morphotype I	3	"I"H1	MK413123 - 125
<i>Trypargilum</i>	<i>T. aurifrons</i>	10	AurH1	MK413152 - 161
<i>Trypargilum</i>	<i>T. agamemnon</i>	3	AgaH1	MK413169
			AgaH2	MK413170
			Sp8H1	MK413171
<i>Trypargilum</i>	<i>T. albitarse</i>	89	AlbH1	MK413013 - 095
			AlbH2	MK413096
			AlbH3	MK413097 - 099
			AlbH4	MK413100 - 101
<i>Trypargilum</i>	<i>T. lactitarse</i>	6	LacH1	MK413146 - 150
			Sp7H1	MK413151
<i>Trypargilum</i>	<i>T. nitidum</i>	6	Nit1H1	MK413162 - 163
			Nit2H1	MK413164 - 165
			Nit2H2	MK413166
			Nit3H1	MK413167
<i>Trypargilum</i>	<i>T. opacum</i>	4	OpaH1	MK413172 - 175
<i>Trypargilum</i>	<i>T. rogenhoferi</i>	11	RogH1	MK413133 - 138
			RogH2	MK413139
			RogH3	MK413140
			RogH4	MK413141
			Sp6H1	MK413142
			Sp6H2	MK413143
<i>Trypoxylon</i>	<i>T. punctivertex</i>	4	Pun1H1	MK413126 - 127
			Pun1H2	MK413128
			Pun2H1	MK413129

<i>Trypoxylon</i>	<i>T. asuncicola</i>	1	AsuH1	MK413102
<i>Nitela</i>	<i>Nitela</i> sp.	1	<i>Nitela</i> sp.	MK413176
<i>Pison</i>	<i>Pison</i> sp.	1	<i>Pison</i> sp.	MK413177

Supplement 4: Samples analysed for *CytB* gene. Indications of subgenus, name given for the morphotype, sequence designation, and sequence identification number in the NCBI/GenBank databases.

Subgenus	Morphotype	Sequence	NCBI/GenBank
<i>Trypargilum</i>	Sp02	Nit1H2	MK413003
<i>Trypargilum</i>	Sp04	Sp6H1	MK413001
<i>Trypargilum</i>	<i>T.</i> sp2	Sp2H1	MK413000
<i>Trypargilum</i>	<i>T.</i> sp4	Sp4H1	MK413002
<i>Trypargilum</i>	Morphotype A	"A"H1	MK413004
<i>Trypargilum</i>	Morphotype B	"B"H1	MK413005
<i>Trypargilum</i>	Morphotype C	"C"H1	MK413006
		"C"H2	MK413007
		"J"H1	MK413008
<i>Trypargilum</i>	Morphotype D	"D"H1	MK413009
<i>Trypoxylon</i>	Morphotype E	"E"H1	MK413010
<i>Trypoxylon</i>	Morphotype H	"H"H1	MK413011
<i>Trypargilum</i>	<i>T. aurifrons</i>	AurH1	MK412977
<i>Trypargilum</i>	<i>T. agamemnon</i>	AgaH1	MK412974
		AgaH2	MK412975
		Sp8H1	MK412976
<i>Trypargilum</i>	<i>T. albitarse</i>	AlbH1	MK412991; MK412994 - 996
		AlbH2	MK412992
		AlbH3	MK412993
<i>Trypargilum</i>	<i>T. lactitarse</i>	LacH1	MK412978
		LacH2	MK412979
		Sp7H1	MK412980
<i>Trypargilum</i>	<i>T. nitidum</i>	Nit1H1	MK412981
		Nit2H1	MK412982 - 983
		Nit3H1	MK412984
<i>Trypargilum</i>	<i>T. opacum</i>	OpaH1	MK412990
<i>Trypargilum</i>	<i>T. rogenhoferi</i>	RogH1	MK412985; MK412987
		RogH2	MK412986; MK412988
		Sp6H2	MK412989
<i>Trypoxylon</i>	<i>T. punctivertex</i>	Pun1H1	MK412997
		Pun1H2	MK412998
		Pun2H1	MK412999
<i>Pison</i>	<i>Pison</i> sp.	<i>Pison</i> sp.	MK413012

Supplement 5: Samples analysed for *pol* gene. Indications of subgenus, name given for the morphotype, sequence designation, and sequence identification number in the NCBI/GenBank databases.

Subgenus	Morphotype	Sequence	NCBI/GenBank
<i>Trypargilum</i>	Sp04	Sp6H1	MK412967
<i>Trypargilum</i>	<i>T. sp2</i>	Sp2H1	MK412966
<i>Trypargilum</i>	Morphotype A	"A"H1	MK412968
<i>Trypargilum</i>	Morphotype B	AgaH2	MK412969
<i>Trypargilum</i>	Morphotype C	"C"H1	MK412970
		"J"H1	MK412971
<i>Trypargilum</i>	Morphotype I	"I"H1	MK412972
<i>Trypargilum</i>	<i>T. aurifrons</i>	AurH1	MK412955
<i>Trypargilum</i>	<i>T. agamemnon</i>	AgaH1	MK412953 - 954
<i>Trypargilum</i>	<i>T. albitarse</i>	AlbH1	MK412950
		AlbH2	MK412951
		AlbH3	MK412952
<i>Trypargilum</i>	<i>T. lactitarse</i>	LacH1	MK412956
		LacH2	MK412957
		Sp7H1	MK412958
<i>Trypargilum</i>	<i>T. nitidum</i>	Nit1H1	MK412959 - 960
<i>Trypargilum</i>	<i>T. rogenhoferi</i>	RogH1	MK412961 - 963
<i>Trypoxylon</i>	<i>T. punctivertex</i>	Pun1H1	MK412964
		Pun1H2	MK412965
<i>Nitela</i>	<i>Nitela sp.</i>	<i>Nitela sp.</i>	MK412973

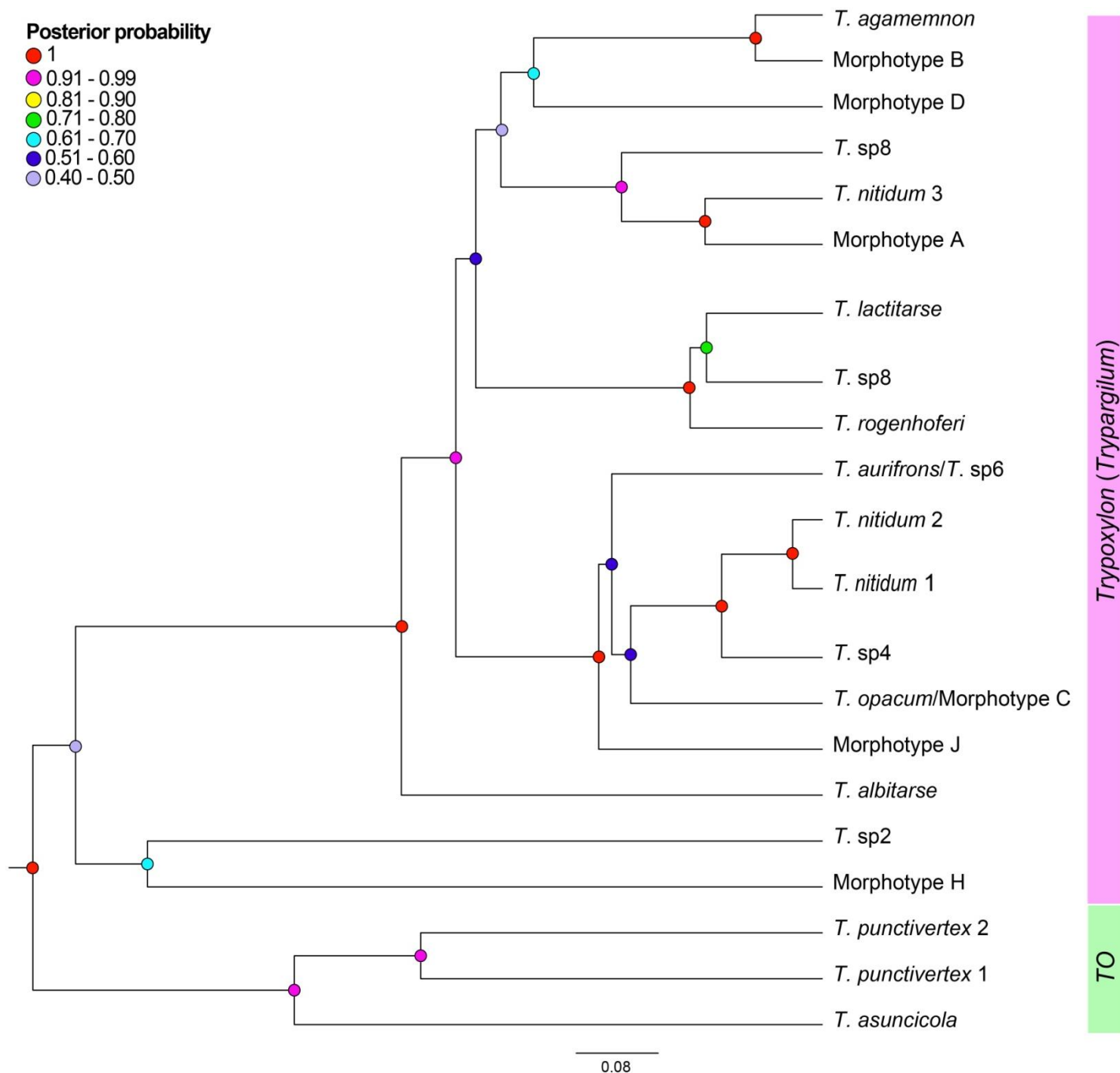
Supplement 6: Information on the sequences selected from databases. Indications of sample subgenus, name given to the morphotype, number of sequences (N) obtained, sequence designation, and sequence identification number in the NCBI/GenBank and BOLD Systems v4 databases.

Subgenus	Species	N	Sequence	BOLD Systems v4	NCBI/GenBank
<i>Trypoxylon</i>	<i>T. attenuatum</i>	11	AttH1	ASGLE1017-10	JN288384
			AttH2	JSJUL1744-11	KR808738
<i>Trypoxylon</i>	<i>T. bidentatum</i>	1	BidH1	BBHYA2762-12	-
<i>Trypoxylon</i>	<i>T. carinatum</i>	6	CarH1	CNROF123-13	KR417862
			CarH2	OPPMO1458-17	-
<i>Trypoxylon</i>	<i>T. clavicerum</i>	25	ClaH1	GBMIN11444-13	KC460790
			ClaH2	GBMIN11492-13	KC460783
			ClaH3	BBHYN095-10	JN294326
<i>Trypargilum</i>	<i>T. collinum</i>	3	ColH1	BBHYA1230-12	-
			ColH2	BBHYA1293-12	-
<i>Trypoxylon</i>	<i>T. figulus</i>	28	FigH1	CNW192-13	-
			FigH2	CNW193-13	-
			FigH3	CNW204-13	-
			FigH4	BBHYL792-10	-
<i>Trypoxylon</i>	<i>T. frigidum</i>	51	FriH1	ASAHY055-12	KM997506
			FriH2	JSYKA053-09	HM395410
			FriH3	JSYKA052-09	HM395409
			FriH4	JSYKA196-10	HM860368
			FriH5	ASGLE999-10	JN289137
			FriH6	ASGLE1000-10	JN288368
			FriH7	JSYKA054-09	HM395411
			FriH8	TWHYM031-09	GU675378
			FriH9	JSHYM804-11	KR804941
			FriH10	BBHYI172-10	JN292291
			FriH11	BBHYJ998-10	JF864624
			FriH12	BBHYI516-10	JN292518
			FriH13	BBHYI173-10	JN292292
			FriH14	PHMTV275-10	JN308036
			FriH15	ERPIR177-12	KR805957
			FriH16	BBHYA2780-12	-
			FriH17	RRMFG2769-15	-
			FriH18	JSYKA592-10	HQ569261
<i>Trypoxylon</i>	<i>T. johnsoni</i>	3	JohH1	ASGLF218-11	KM996017
			JohH2	ASGLF219-11	KM996525
			JohH3	TTMHY667-11	KR883303
<i>Trypargilum</i>	<i>T. lactitarse</i>	2	LacH1	TTHYW021-08	KR874003
<i>Trypoxylon</i>	<i>T. lissonotum</i>	1	LisH1	HYSAF086-12	-
<i>Trypoxylon</i>	<i>T. medium</i>	1	MedH1	FBACA948-10	HQ947977
<i>Trypoxylon</i>	<i>T. minus</i>	1	MinH1	ARTNO183-17	-

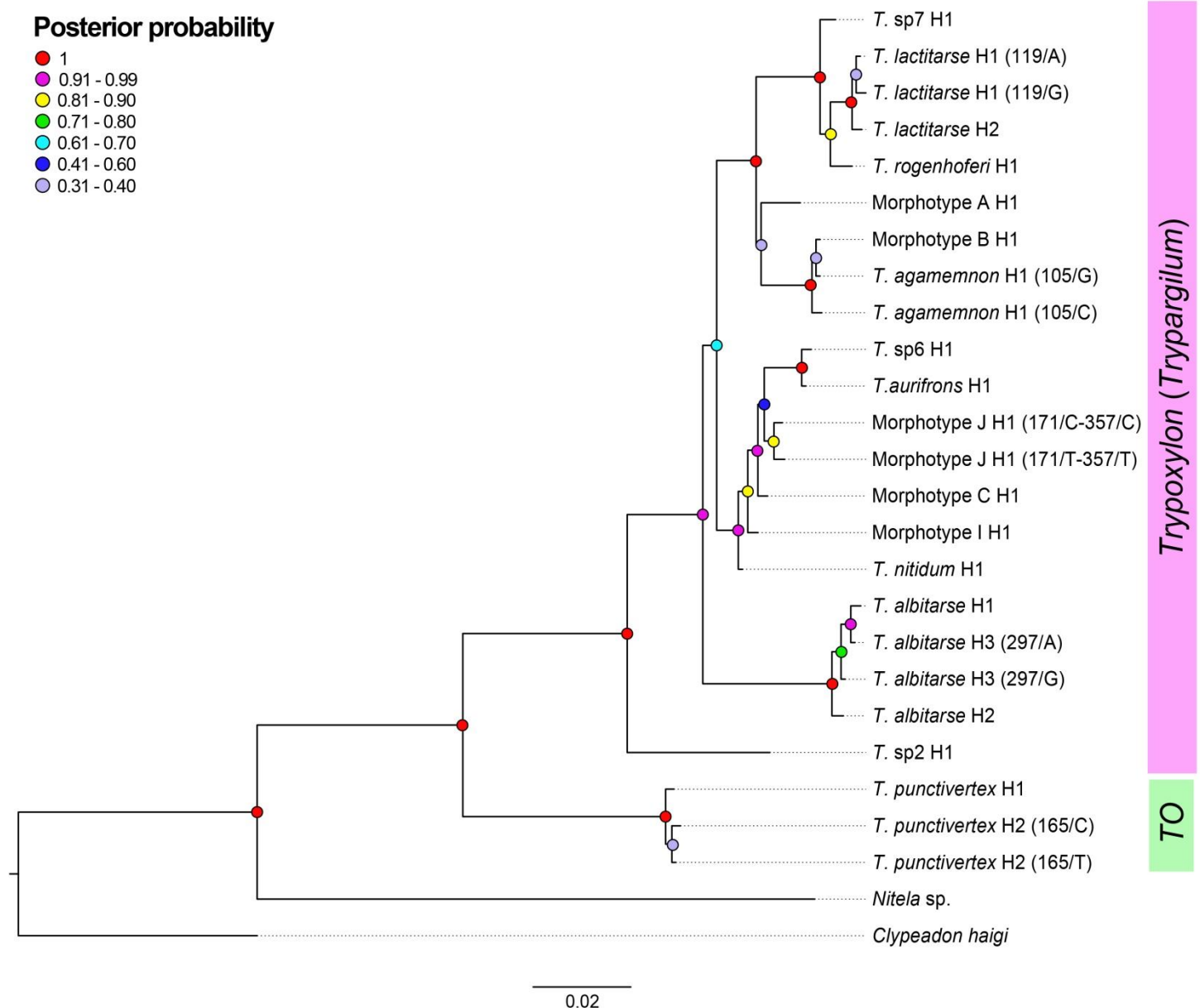
<i>Trypargilum</i>	<i>T. nitidum</i>	10	NitH1	BBHYA3337-12	-
			NitH2	BBHYA850-12	-
			NitH3	CNPPF1230-12	KJ166704
			NitH4	CNPPE1221-12	KJ090765
			NitH5	SYC1487-14	KX055172
			NitH6	OPPEO6415-17	-
			NitH7	OPPQG256-17	-
<i>Trypoxylon</i>	<i>T. pennsylvanicum</i>	10	PenH1	ASGLE1649-10	JN288868
			PenH2	OPPOC1344-17	-
			PenH3	CNPPG203-12	KJ163327
<i>Trypargilum</i>	<i>T. politum</i>	10	PolH1	BBHYA336-12	-
			PolH2	TTHYW902-08	KR895965
			PolH3	BBHY338-12	-
			PolH4	BBHYA414-12	-
			PolH5	BBHYA570-12	-
			PolH6	OPPFS130-17	-
			PolH7	OPPQQ074-17	-
<i>Trypargilum</i>	<i>T. spinosum</i>	5	SpiH1	BBHYA1228-12	-
			SpiH2	BBHYA1248-12	-
			SpiH3	BBHYA1306-12	-
			SpiH4	BBHYA1307-12	-
			SpiH5	GMMCF066-14	-
<i>Trypoxylon</i>	<i>T. stroudi</i>	1	StrH1	HYSAF121-12	-
<i>Trypargilum</i>	<i>T. tridentatum</i>	1	TriH1	BBHYA1294-12	-
<i>Clypeadon</i>	<i>C. haigi</i>	1	Clypeadon	-	AY945104.1

Supplement 7: Optimal threshold (OT) estimated using barcoding gap value and the "localMinima" function of the SPIDER package.

Genectic distance approach	With outgroups	Without outgroups
Considering all COI sequences (n = 333)	3.81%	3.88%
Considering a sequence per haplotype (n = 102)	4.34%	4.83%



Supplement 8: Species tree of the genus *Trypoxylon* generated by Bayesian Inference based on mitochondrial genes *COI* and *CytB*. TO indicates the subgenus *Trypoxylon* (*Trypoxylon*).



Supplement 9: Phylogenetic tree of *Trypoxylon* generated by Bayesian Inference using sequences of the *pol* gene. TO indicates the subgenus *Trypoxylon* (*Trypoxylon*) and the information inside parentheses indicates both nucleotides observed at each position in the sequences.

References

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