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Studies on the genus *Thysananthus* (Marchantiophyta, Lejeuneaceae) 3. Terpenoid chemistry and chemotaxonomy of selected species of *Thysananthus* and *Dendrolejeunea fruticosa*

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Abstract – Gas chromatography and mass spectrometry of 20 samples of *Thysananthus* (*T. comosus, T. convolutus, T. retusus, T. spathulistipus*) and one of *Dendrolejeunea* (*D. fruticosa*) from Malaysia and Thailand revealed the presence in most taxa of large quantities of sesquiterpenoids and unidentified diterpenoids. Isolepidozene was detected as a major component in *T. comosus*, *T. convolutus* and *T. spathulistipus*, pinguisane sesquiterpenoids as major components in *T. retusus*, and the unidentified $(M^+)304$, 271(100), 105(90) as the most abundant component of *D. fruticosa*. The large chemical heterogeneity detected in *T. convolutus* correlates with the variation in leaf dentation observed in the species and supports the resurrection of *T. gottschei*, previously considered a synonym of *T. convolutus*. The subdivision of *Thysananthus* into two subgenera, subg. *Thysananthus* and subg. *Sandeanthus*, and the treatment of *Dendrolejeunea* as a separate genus, are chemically supported.

***Dendrolejeunea fruticosa* / diterpenoids / GC-MS / liverwort / sesquiterpenoids / *Thysananthus comosus* / *Thysananthus convolutus* / *Thysananthus retusus* / *Thysananthus spathulistipus* / volatiles**

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INTRODUCTION

Liverworts are able to synthesize large quantities of terpenoids, especially sesquiterpenoids, diterpenoids and lipophilic aromatic compounds, which are stored in oil bodies (e.g., Gradstein *et al.*, 1985). The Lejeuneaceae are the largest family of liverworts with about 1200 species in 78 genera (Frey & Stech, 2009, with updates). Several studies have been conducted on the terpenoids and aromatic compounds of Lejeuneaceae (e.g. Asakawa, 1982, 1995, 2004; Asakawa *et al.*, 1980a, 1980b; Gradstein *et al.*, 1981, 1985, 1988; Kruijt *et al.*, 1986). These substances, especially terpenoids, often show strong biological activity and may play an important role in the defence of the plants against environmental hazards (Gradstein, 1994) or predators (Harinantenaina *et al.*, 2006). The above-mentioned studies also identified some substances as taxonomic markers for members of the family.

Thysananthus Lindenb. is a member of Lejeuneaceae subfamily Ptychanthoideae Mizut. (Mizutani, 1961; Gradstein, 1975, 1994). The genus is characterized by (1) purely *Lejeunea* type vegetative branches, (2) ventral merophyte 4-12 cells wide, (3) stem epidermis cells not larger than medullary cells, with thick, brownish walls, (4) leaves convolute when dry, usually toothed, (5) leaf cells elongate, (6) gynoecial innovations with lejeuneoid leaf sequence, and (7) perianth with 3(-10) keels (Thiers & Gradstein, 1989; Gradstein, 1992; Gradstein *et al.*, 2002; Sukkharak & Gradstein, 2010a). Thus defined, the genus contains ten species rowing as epiphytes in moist lowland forests and montane cloud forests throughout the tropics (Sukkharak & Gradstein, 2010a). The centre of diversity is in Southeast Asia with five species (*T. aculeatus* Herzog, *T. comosus* Lindenb., *T. convolutus* Lindenb., *T. retusus* (Reinw. *et al.*) B. Thiers *et* Gradst., *T. spathulistipus* (Reinw. *et al.*) Lindenb.). *Thysananthus spathulistipus* also reaches to tropical Africa. Three species are found in New Guinea and the Solomon Islands (*T. discretus* Sukkharak *et* Gradst., *T. mollis* Steph., *T. appendiculatus* Steph.) and one is only known from Papua New Guinea (*T. montanus* Gradst. *et al.*). Finally, one species, *T. amazonicus* (Spruce) Schiffn., is known from the New World tropics. The species of *Thysananthus* are attributed to the two subgenera: subg. *Thysananthus* (*T. aculeatus, T. amazonicus, T. appendiculatus, T. comosus, T. convolutus, T. discretus, T. mollis, T. montanus, T. spathulistipus*) and subg. *Sandeanthus* B. Thiers *et* Gradst. (*T. retusus*) (Thiers & Gradstein, 1989; Gradstein, 1992, with updates).

Dendrolejeunea is a monotypic genus, containing only *D. fruticosa* (Lindenb. *et* Gottsche) Lacout. from tropical Asia, northern Australia and the Pacific region. First described by Spruce (1884) as a subgenus of *Lejeunea*, *Dendrolejeunea* was long included in *Thysananthus* but was resurrected as a separate genus by Gradstein (1992). Morphologically, *Dendrolejeunea* stands out by its dendroid habit, having creeping stoloniform stems and upright, regularly pinnate branches. In contrast, members of *Thysananthus* are never dendroid and share projecting growth and irregularly pinnate branching. Molecular data have revealed that *Dendrolejeunea* is the sister group of *Thysananthus* (Wilson *et al.*, 2007).

Seven species of *Thysananthus*: *T. amazonicus*, *T. appendiculatus*, *T. convolutus*, *T. discretus* (under *T. convolutus* sample nr. 49 in Gradstein *et al.*, 1985), *T. mollis*, *T. retusus* (under *T. fruticosus* in Gradstein *et al.*, 1985) and *T. spathulistipus*, have been screened for the occurrence of terpenoids, flavonoids, sterols, and aromatic compounds (Gradstein *et al.*, 1985; Kruijt *et al.*, 1986; Harinantenaina *et al.*, 2006). The results showed the presence of pinguisane sesquiterpenoids, clerodane diterpenoids and unidentified diterpenoids.

As part of a world-wide revision of *Thysananthus* by the first author, we hereby report the results of chemical analysis of four species of *Thysananthus* (*T. comosus*, *T. convolutus*, *T. retusus*, *T. spathulistipus*) and of the closely related *Dendrolejeunea fruticosa.*

MATERIAL AND METHODS

Plant material

Twenty samples of *Thysananthus* and one sample of *Dendrojeunea* were collected mainly by the first author and her associates in Malaysia and Thailand in 2009 (Tab. 1). Dried voucher specimens were deposited in BKF and GOET.

Extraction and analysis

Plant material (0.25 g of each species) was purified, air-dried and crushed in mortal to receive fine powder. The powdered material was extracted three times with diethyl ether (3 × 3 ml) at room temperature, and the crude extract was filtered through a Pasteur pipette packed with Celite. The filtered extract was dried using anhydrous sodium sulfate as a drying agent, then evaporated to receive 100 µl of extract. One µl of the crude extract was analyzed by GC-MS using an Agilent Technologies 6890N gas chromatograph coupled with a mass selective detector (Agilent Technologies 5973), on an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). Oven temperature was 50°C with 3 minutes initial hold, and then to 250°C temperature programmed at 5°C/min, and 15 minutes at 250°C. Injection temperature was 280°C and helium (1ml/min) was used as a carrier gas. The detector was operated in electron impact mode (70eV with 3 scans/s and mass range *m/z* 40-500) at 230°C. Each extract was analyzed three times. The retention indices were calculated relative to C9-C30 *n*-alkanes. Compounds were identified using a computer-supported spectral library (Hochmuth, 2008), mass spectra of reference compounds, as well as MS data from the literature (Joulain & König, 1998; Linstrom & Mallard, 2001) and the library database of the Faculty of Pharmaceutical Sciences, Tokushima Bunri University. Compound identities were confirmed by comparison of retention indices with reference compounds and published data (Linstrom & Mallard, 2001). Quantification was done based on peak area.

RESULTS AND DISCUSSION

In total, 66 different sesquiterpenoids and diterpenoids were detected in the five investigated species (21 samples). Of these, about two-thirds (42) are unidentified compounds. The taxonomic distribution and abundance of the identified and unidentified compounds are shown in Table 2, chemical structures of identified components are depicted in Figure 1. Numbers in bold face in the text refer to the compound figure numbers. The chemotaxonomic relevance of the detected compounds is discussed below.

Table 1. Voucher information and sample number

Taxon	*Sample number*	*Geographical origin*	*Collection date*	*Voucher number and herbarium*
Thysananthus comosus Lindenb.	1	Pangkor Island, Malaysia	4 Mar 2009	*Sukkharak 730* (BKF, GOET)
	2	Khao Luang National Park, Thailand	24 Feb 2009	*Chantanaorrapint s.n.* (PSU)
	3	Sri Pang Nga National Park, Thailand	12 Jul 2008	*Chantanaorrapint 2121* (PSU)
	4	Ton Nga Chang waterfall, Songkhla Province, Thailand	17 Aug 2009	*Inuthai s.n.* (PSU)
Thysananthus convolutus Lindenb.	1	Genting Highland, Malaysia	3 Mar 2009	*Sukkharak 717* (BKF, GOET)
	2	Genting Highland, Malaysia	3 Mar 2009	*Sukkharak 718* (BKF, GOET)
	3	Genting Highland, Malaysia	3 Mar 2009	*Sukkharak 720* (BKF, GOET)
	4	San Yen, Khao Nan National Park, Thailand	10 Mar 2009	*Sukkharak 769* (BKF, GOET)
	5	San Yen, Khao Nan National Park, Thailand	11 Mar 2009	*Sukkharak 803* (BKF, GOET)
Thysananthus retusus (Reinw. *et al.*) B. Thiers *et* Gradst.	1	San Yen, Khao Nan National Park, Thailand	19 Feb 2009	*Chantanaorrapint s.n.* (PSU)
	2	San Yen, Khao Nan National Park, Thailand	10 Mar 2009	*Sukkharak 733* (BKF, GOET)
	3	San Yen, Khao Nan National Park, Thailand	10 Mar 2009	*Sukkharak 741* (BKF, GOET)
	4	San Yen, Khao Nan National Park, Thailand	10 Mar 2009	*Sukkharak 743* (BKF, GOET)
Thysananthus spathulistipus (Reinw. *et al.*) Lindenb.	1	San Yen, Khao Nan National Park, Thailand	10 Mar 2009	*Sukkharak 739* (BKF, GOET)
	2	San Yen, Khao Nan National Park, Thailand	10 Mar 2009	*Sukkharak 751* (BKF, GOET)
	3	San Yen, Khao Nan National Park, Thailand	10 Mar 2009	*Sukkharak 752* (BKF, GOET)
	4	San Yen, Khao Nan National Park, Thailand	10 Mar 2009	*Sukkharak 762* (BKF, GOET)
	5	San Yen, Khao Nan National Park, Thailand	10 Mar 2009	*Sukkharak 768* (BKF, GOET)
	6	San Yen, Khao Nan National Park, Thailand	10 Mar 2009	*Sukkharak 788* (BKF, GOET)
	7	San Yen, Khao Nan National Park, Thailand	11 Mar 2009	*Sukkharak 793* (BKF, GOET)
Dendrolejeunea fruticosa (Lindenb. *et* Gottsche) Lacout.	1	Sri Pang Nga National Park, Thailand	12 Jul 2008	*Chantanaorrapint 2112* (PSU)

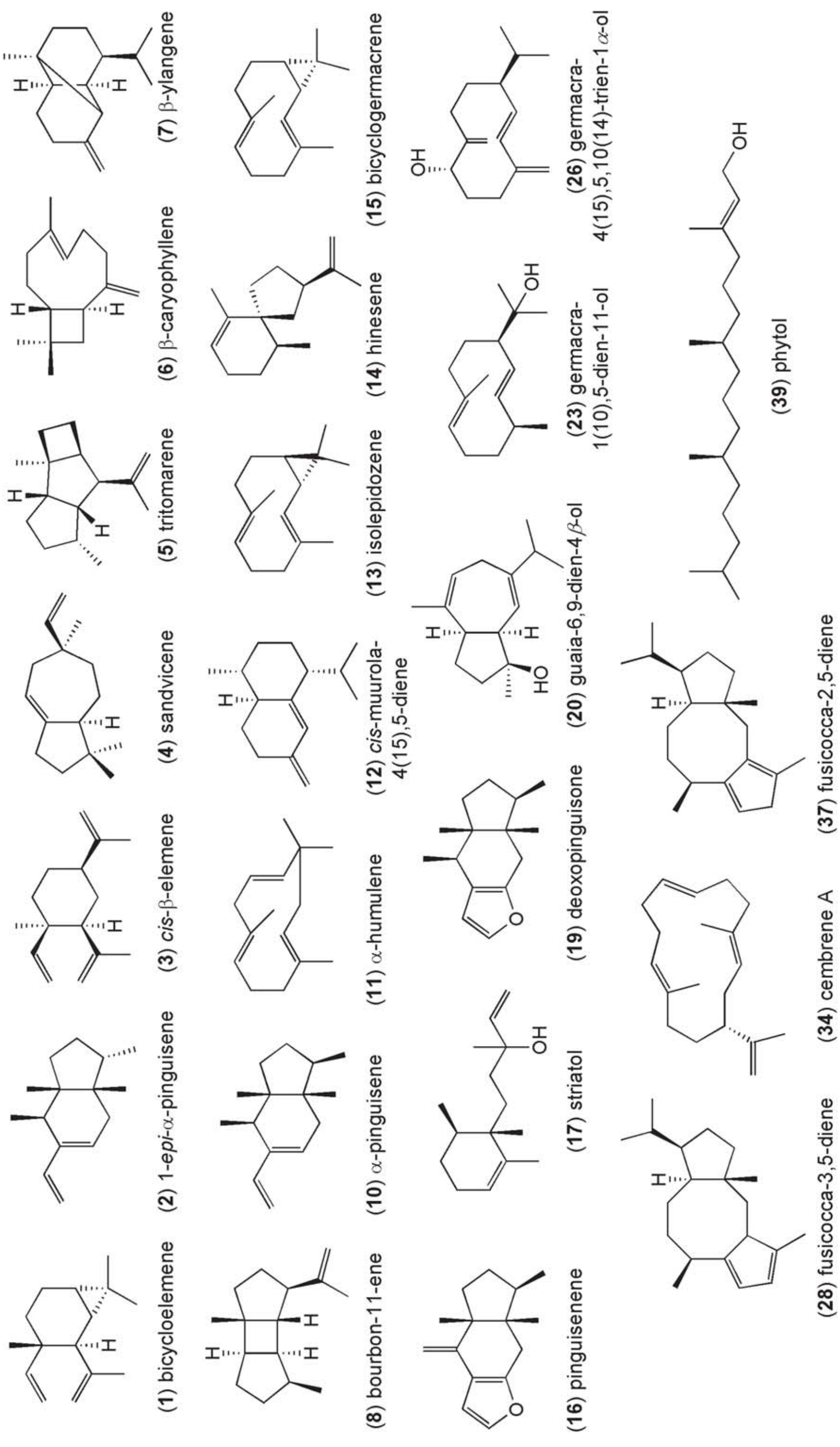

Fig. 1. Chemical structure of sesquiterpenoids (**1-8**, **10-17**, **19-20**, **23**, **26**) and diterpenoids (**28**, **34**, **37**, **39**) identified in selected *Thysananthus* species and *Dendrolejeunea fruticosa*.

Table 2. The distribution of the sesqui- and diterpenoids detected in the analyzed samples of *Thysananthus* species and *Dendrolejeunea fruticosa* (Lindenb. *et* Gottsche) Lacout. RRI - relative retention indices calculated against *n*-alkanes on HP-5MS column; tr – traces (< 0.2%)

No	***Compounds***	***RRI***	*T. comosus*				*T. convolutus*					*T. retusus*				*T. spathulistipus*							*D. fruticosa*
			1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**1**
1	Bicycloelemene	1336	0.8			0.6	0.5	0.4	1.3											10.5		0.6	
2	1-*epi*-α-Pinguisene	1367										0.2	0.2	tr	tr	0.2		tr					
3	*cis*-β-Elemene	1377	3.3	1.9	2.2	14.2	0.3	0.3	1.7	1.7	3.1					0.2			0.7	0.4	0.8	0.5	0.2
4	Sandvicene	1399										0.2	0.2	tr	tr								
5	Tritomarene	1416								1.0	1.4												
6	β-Caryophyllene	1423																	3.2		4.5		
7	β-Ylangene	1431	0.8		0.2	1.2	0.2	0.2	0.3	0.5	0.6					0.3	0.5	0.2		0.2		1.4	
8	Bourbon-11-ene	1435								2.4	3.5												
9	(M^+)204, 107(100), 93(62)	1438																					2.4
10	α-Pinguisene	1444										12.4	9.9	7.3	6.5	0.9		0.5	1.0		1.6	1.5	
11	α-Humulene	1452																		3.2			
12	*cis*-Muurola-4(15),5-diene	1462	5.1			7.7						0.6	0.5	0.5	0.5	0.4	0.7	0.8	0.3		3.8	9.0	
13	Isolepidozene	1483	18.4	7.9	7.0	21.2	10.7	9.1	22.3	12.6	21.5	1.0	1.4	2.4	1.8	12.3	26.3	8.6	19.6	11.7	21.5	26.0	1.1
14	Hinesene	1494															6.1						
15	Bicyclogermacrene	1495	2.3			3.1	2.4	2.3	3.4							5.1				21.0		3.7	
16	Pinguisenene	1544													tr			0.6			0.4	0.4	
17	Striatol	1550														1.0		tr					
18	(M^+)208, 137(100), 109(78)	1571														4.5		3.9	2.0				
19	Deoxopinguisone	1579										tr	tr	1.0	15.1			1.4	4.3		3.1	16.2	
20	Guaia-6,9-dien-4*β*-ol	1582	0.3		3.1												0.3			2.6			8.4
21	(M^+)216, 82(100), 93(62)	1593										4.1	3.5	3.0	3.0								
22	(M^+)222, 82(100), 41(68)	1632		0.2	2.1												1.0	0.6	0.9				
23	Germacra-1(10),5-dien-11-ol	1653	0.4			0.3										0.5	0.8	0.5	0.6		0.8	0.9	
24	(M^+)220, 107(100), 43(80)	1661	1.0	1.1	1.9	0.4										2.9	1.1	2.1	2.5		3.7	2.3	
25	(M^+)222, 95(100), 109(56)	1679										1.9	1.7	1.5	0.2			0.8					
26	Germacra-4(15),5,10(14)-trien-1*α*-ol	1680		tr	2.1																		
27	(M^+)235, 109(100), 121(40)	1831										7.5	6.9	6.9	1.4	7.0		7.3	3.3		2.1	1.5	
28	Fusicocca-3,5-diene	1850										0.5	0.4	0.6	0.7								
29	(M^+)234, 95(100), 124(48)	1928														2.6		3.8	2.1		2.9	1.7	
30	(M^+)222, 109(100), 169(20)	1936										1.8	1.7	1.3	0.3	1.5		2.0					
31	(M^+)232, 217(100), 109(26)	1942																	3.0			0.4	
32	(M^+)272, 257(100), 91(40)	1949																					2.0
33	(M^+)272, 257(100), 80(45)	1953						12.2			0.3					4.0	0.4						

Table 2. The distribution of the sesqui- and diterpenoids detected in the analyzed samples of *Thysananthus* species and *Dendrolejeunea fruticosa* (Lindenb. *et* Gottsche) Lacout. RRI - relative retention indices calculated against *n*-alkanes on HP-5MS column; tr – traces (< 0.2%) *(continued)*

No	***Compounds***	***RRI***	*T. comosus*				*T. convolutus*					*T. retusus*				*T. spathulistipus*							*D. fruticosa*
			1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**1**
34	Cembrene A	1962														1.1		2.3		1.6			
35	(M^+)274, 124(100), 137(24)	1977										1.9	2.3	3.1	1.1								
36	(M^+)232, 175(100), 161(50)	1986										1.7	1.6	1.5	0.7	2.0		2.8	0.7		0.9	0.6	
37	Fusicocca-2,5-diene	2020										6.8	2.3	2.5	4.8								2.9
38	(M^+)290, 43(100), 81(75)	2068														1.2	1.6						
39	Phytol	2114		0.5	1.9																		
40	(M^+)302, 190(100), 81(95)	2182	14.1				42.1	33.8	16.9	tr													
41	(M^+)288, 255(100), 105(70)	2187																					1.9
42	(M^+)288, 273(100), 91(60)	2239																					0.4
43	(M^+)302, 207(100), 189(48)	2245	1.1				2.5	1.9	0.7								0.9						
44	(M^+)302, 81(100), 95(60)	2254	1.4	9.3		3.8	7.6	6.6	3.9			1.9	3.4	3.2	3.7		0.7						
45	(M^+)304, 147(100), 161(60)	2275										2.2	2.6	2.5	1.2								
46	(M^+)290, 95(100), 189(90)	2279		1.6	1.6					4.0	0.9						1.0						
47	(M^+)302, 81(100), 185(60)	2342								10.8	44.3												
48	(M^+)302, 95(100), 81(82)	2358															18.0						
49	(M^+)290, 81(100), 55(90)	2406								4.6													
50	(M^+)302, 302(100), 91(50)	2423																					1.8
51	(M^+)318, 81(100), 206(98)	2432										6.0	18.3	25.4	32.2								
52	(M^+)316, 221(100), 81(35)	2434	1.6	4.3		1.5	3.4	1.8	0.8							1.1							
53	(M^+)318, 125(100), 81(38)	2441								3.3						1.0		9.7		23.7			
54	(M^+)304, 271(100), 105(90)	2449																					21.1
55	(M^+)320, 207(100), 81(83)	2489					7.0	4.7	1.7								11.3						
56	(M^+)318, 81(100), 95(58)	2516																	3.5				
57	(M^+)332, 81(100), 237(80)	2522	2.3													2.2							
58	(M^+)334, 319(100), 123(40)	2560					1.2	1.6	2.9														
59	(M^+)318, 303(100), 123(50)	2584					1.1	0.9	1.7														
60	(M^+)360, 81(100), 43(80)	2586																	5.0		5.2	6.0	
61	(M^+)360, 81(100), 43(90)	2601																	2.9		2.5	2.3	
62	(M^+)374, 43(100), 81(58)	2687																	2.9		2.6	3.6	
63	(M^+)318, 207(100), 189(60)	2858		7.7	7.0	3.6																	
64	(M^+)318, 189(100), 41(20)	2864								3.8													
65	(M^+)318, 151(100), 303(32)	2895		18.3	9.7	14.2																	
66	(M+)318, 119(100), 134(60)	2923		2.9	4.6	1.6																	

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Thysananthus comosus Lindenb.

Three compounds were detected in all four samples of *Thysananthus comosus*: *cis*-β-elemene (**3**), isolepidozene (**13**) and the unidentified $(M^+)220$, 107(100), 43(80). The Malaysian specimen of this species (sample 1) produces large amounts of $(M^+)302$, 190(100), 81(95) which was also detected as a major component in *T. convolutus* from Malaysia. Samples 2-4 from Thailand produce three unidentified compounds $(M^+)318$, 207(100), 189(60), $(M^+)318$, 151(100), 303(32) and $(M^+)318$, 119(100), 134(60), which have not been detected in any other analyzed *Thysananthus* species. In ecological respect, the samples of *T. comosus* are quite distinct. Sample 1 is from rock in coastal forest at the sea shore, whereas the other samples are from inland forests. We suggest that the deviating chemical composition of sample 1 might be due to stress, induced by the salt-spray environment. The same phenomenon is observed in the widespread holarctic *Conocephalum conicum* (L.) Dumort. which is made up of different geographical and chemical races. *Conocephalum conicum* type I is widely distributed while type II occurs in coastal locations (Wood *et al.*, 1996). GC-mass-spectrometric analysis of 280 samples of *C. conicum* revealed that type I elaborates (–)-sabinene as a major compound whereas type II produces large amounts of (+)-bornyl acetate (Toyota *et al.*, 1997). Kim *et al.* (2001) studied the phylogenetic relationships among the two chemotypes of *C. conicum* using *psbA* sequences. Their results showed that the *psbA* sequences of *C. conicum* type I and II are identical, suggesting that they are conspecific. These finding indicate that the detected chemical differences within *C. conicum* are environmentally controlled, as has been suggested by Asakawa (1995) and Harinantenaina & Asakawa (2004). In *T. comosus*, however, study of a larger number of samples is needed to corroborate the observed correlation between chemical constitution and habitat.

Thysananthus convolutus Lindenb.

In all samples of *T. convolutus* relatively large amounts of isolepidozene (**3**) were detected. However, the samples from Malaysia (1-3) and Thailand (4, 5) are chemically different. Large amounts of the unidentified diterpenoid $(M^+)302$, 190(100), 81(95) were detected in the Malaysian samples whereas those from Thailand produce large amounts of $(M^+)302$, 81(100), 185(60), which was absent in the samples from Malaysia. The chemical dissimilarity of the samples of *T. convolutus* is reflected in the morphology, as the samples from Malaysia have entire leaves, whereas Thai samples have toothed leaves. *Thysananthus convolutus* is a polymorphic species with respect to the dentation of leaves, underleaves and female bracts, which varies from strongly dentate to edentate. The edentate form has been called *T. gottschei* (Jack *et* Steph.) Steph., which was reduced to a synonym of *T. convolutus* (Grolle & Piippo, 1984). Our data suggest that *T. gottschei* may have to be resurrected as a separate taxon. This taxonomic issue will be addressed in a more broadly-based, monographic study (Sukkharak, in prep.).

Thysananthus retusus (Reinw. *et al.*) B. Thiers *et* Gradst.

Pinguisane sesquiterpenoids are detected as major compounds in all 4 samples of *T. retusus*. The presence of α-pinguisene (**10**) and deoxopinguisone (**19**), reported earlier from *T. retusus* (under *T. fruticosus*) by Gradstein *et al.*

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(1985), is confirmed. Another abundant compound occurring only in this species is an unidentified diterpenoid (M^+)318, 81(100), 206(98). The chemical constitution of the samples of *T. retusus* is rather different from that of the other analyzed *Thysananthus* species. The chemical data are supportive of the classification of *Thysananthus* into 2 subgenera, subg. *Thysananthus* and subg. *Sandeanthus*, based on morphology (Thiers & Gradstein, 1989; Gradstein, 1992). *T. mollis* and *T. planus* Sande Lac. (= *T. retusus*) were earlier placed in section *Vittatae* by Verdoorn (1934), together with *Dendrolejeunea fruticosa* (= *T. fruticosus*), but this section was broken up by Thiers & Gradstein (1989). Morphologically, *Thysananthus mollis* and *T. retusus* share the presence of a vitta in the leaves and appendages on the leaf lobules (Sukkharak & Gradstein, 2010b). Chemically, however, the two species seem to be quite different, *T. mollis* being poor in sesquiterpenoids (Gradstein *et al.*, 1985).

***Thysananthus spathulistipus* (Reinw. *et al.*) Lindenb.**

In all analyzed samples of *Thysananthus spathulistipus* quite large amounts of isolepidozene (**13**) were found. Five from the seven samples produce pinguisane sesquiterpenoids. Samples 3 and 5, and also sample 1 (small amount), biosynthesize the unidentified diterpenoid (M^+)318, 125(100), 81(38). Compounds (M^+)360, 81(100), 43(80), (M^+)360, 81(100), 43(90) and (M^+)374, 43(100), 81(58) are abundantly present in samples 4, 6 and 7. Sample 2 differs from the others by the presence of large amounts of compounds (M^+)302, 95(100), 81(82) and (M^+)320, 207(100), 81(83). The latter compound is also abundantly present in the Malaysian samples of *T. convolutus*. Morphologically, however, sample 2 fits *T. spathulistipus* as currently conceived. The data suggest that *T. spathulistipus* is chemically a rather variable species.

***Dendrolejeunea fruticosa* (Lindenb. *et* Gottsche) Lacout.**

The investigated sample of *Dendrolejeunea fruticosa* differs chemically from the analyzed *Thysananthus* species by the presence of the sesquiterpenoid alcohol guaia-6,9-dien-4β-ol (**20**) and an unidentified diterpenoid (M^+)304, 271(100), 105(90) as the two most abundant components. Interestingly, fusicocca-2,5-diene (**37**) detected in all samples of *T. retusus* was also found in the sample of *D. fruticosa*.

CONCLUSIONS

The chemotaxonomic conclusions presented here are still preliminary. Noteworthy is the chemical heterogeneity of *Thysananthus convolutus*, which is congruent with the morphological variation in this species, viz. the dentation of leaves, underleaves and female bracts. In other instances, however, the chemical findings do not coincide with morphology. For instance, unidentified compound (M^+)302, 190(100), 81(95) was detected in *T. convolutus* and in one sample (1) of *T. comosus*, and the unidentified (M^+)320, 207(100), 81(83) in *T. spathulistipus* sample 2 and Malaysian *T. convolutus* but not in other samples of these species.

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Furthermore, pinguisane sesquiterpenoids were only found in the morphologically very different species *T. retusus* and *T. spathulistipus*. The separation of *Dendrolejeunea* from *Thysananthus* was confirmed by the very different chemical composition of *Dendrolejeunea fruticosa* detected in this study. The results obtained in this study will be substantiated by isolation and identification of the detected unidentified compounds, which is in progress (Ludwiczuk, in prep.), and by molecular-phylogenetic analysis of DNA markers and further morphological study (Sukkharak *et al.*, submitted).

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