Karyomorphological studies of six species of *Lachenalia* (Asparagaceae)*

Shuichi Hamatani[1)] and Norikazu Tagashira[2)]

ラケナリア属（キジカクシ科）６種における核形態学的研究

濱谷修一[1)]・田頭紀和[2)]

Summary

Chromosome studies in six species of *Lachenalia* were made. The chromosome numbers of 2n=14, 18 or 28 were counted. Chromosome number of *L. angelica* (2n=14) was reported here for the first time, and of *L. attenuata* (2n=18) and *L. youngii* (2n=18) were different from the numbers of previous reports.

Keywords: Karyotype, Chromosome number, *Lachenalia, Lachenalia angelica, Lachenalia attenuata, Lachenalia youngii*

Introduction

Lachenalia is an ornamentally meritorious genus in the family Asparagaceae. This genus consists of 133 species (139 taxa), and is endemic to South Africa and Namibia (Duncan 2012). In *Lachenalia*, chromosome studies have been done in 94 species (Duncan 2012), and demonstrated their chromosome numbers of 2n=10, 12, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 32, 36, 40, 42, 44, 49 and 56 (e.g. Mofett 1936, de Wet 1957, Fernandes and Neves 1962, Crosby 1986, Johnson and Brandham 1997, Hamatani *et al.* 1998, 2004, 2007, Spies *et al.* 2000, 2002, 2008, 2009, Spies 2004 and Hamatani 2011). Based on the previous information of their chromosome numbers, the basic chromosome number of this genus have been concluded (Spies *et al.* 2011), and detail karyomorphological studies were held on some reports (e.g. Mofett 1936, Hamatani *et al.* 1998, 2004, 2007, 2009, 2010, Spies 2004 and Hamatani 2011). However, many of the previous information were remitted only their chromosome number, therefore there are still remaining the necessities for detail observations about the chromosome character of *Lachenalia* species.

In this study, we made cytogenetical observations on six species of *Lachenalia* for the first time.

Material and Methods

Plant species observed in this study are listed in Table 1. The material plants were cultivated in the Hiroshima Botanical Garden.

For the chromosome observation, root tips were harvested and pretreated in 2mM 8-hydroxyquinoline at 20℃ for two hours, before they were fixed and stored in the 3:1 ethanol and acetic acid below freezing

---

* Contribution from the Hiroshima Botanical Garden No.105

1) The Hiroshima Botanical Garden

2) Department of Human Life and Environment, Faculty of Human Life Studies, Hiroshima Jogakuin University.

Bulletin of the Hiroshima Botanical Garden, No.34:1-8, 2019.

Table 1. Chromosome numbers of six species of *Lachenalia* studied

Species	Section*	Chromosome number (2n)		Reference
		Present count	Previous count	
Lachenalia angelica W. F. Barker ex G. D. Duncan	Latae	14	–	(New Count)
Lachenalia attenuata W. F. Barker ex G. D. Duncan	Oblongae	18	14	Spies *et al.* 2009
Lachenalia cernua G. D. Duncan	Urceolatae	28	28	Spies *et al.* 2008
Lachenalia minima W. F. Barker	Oblongae	18	18	Spies *et al.* 2008
Lachenalia ventricosa Schltr. ex W. F. Barker	Lachenalia	14	14	Spies *et al.* 2008
Lachenalia youngii Baker	Oblongae	18	16	Spies *et al.* 2008

* Referred from Duncan 2012

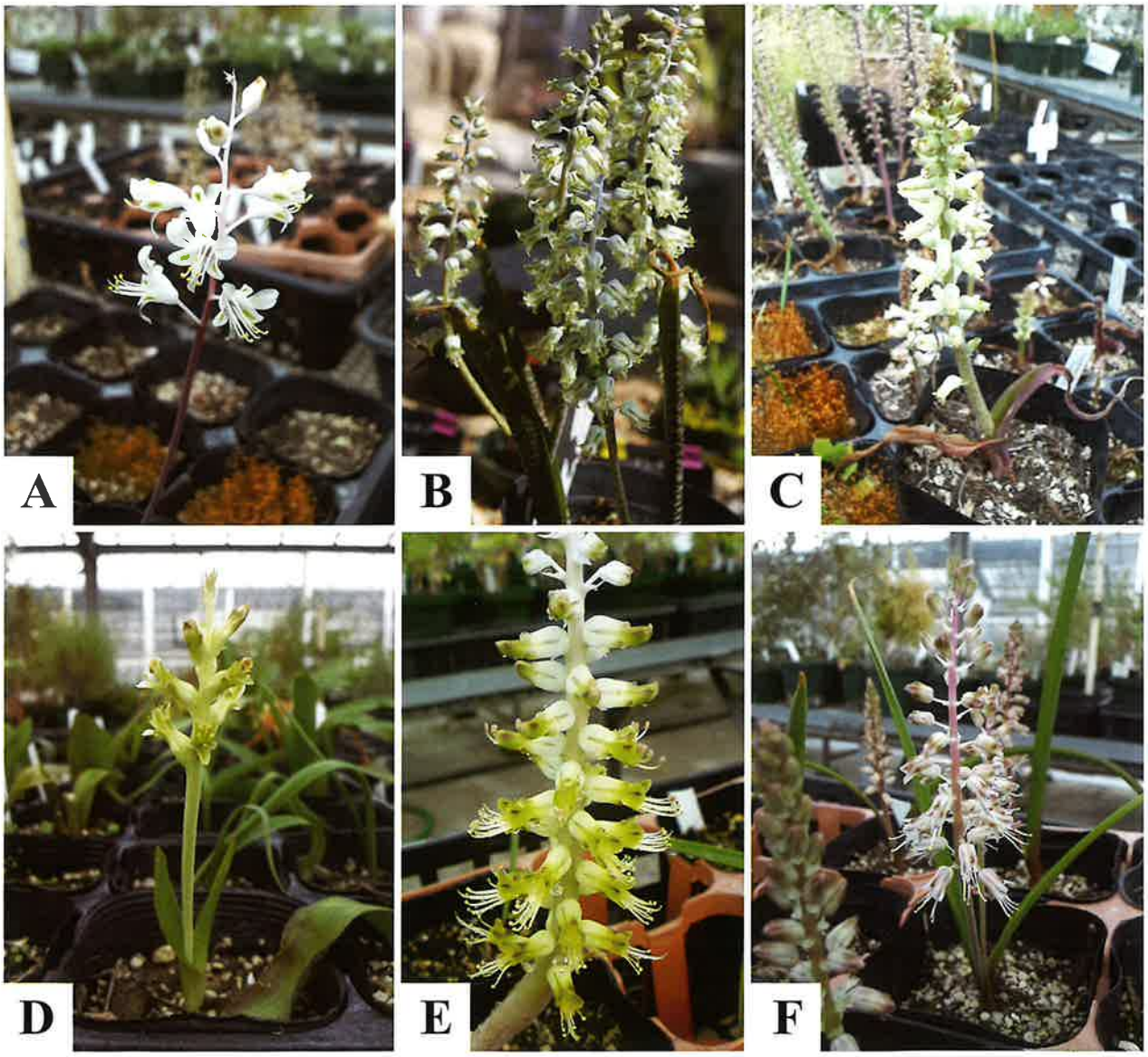

Fig. 1. Flowers of *Lachenalia* studied. A: *L. angelica*, B: *L. attenuata*, C: *L. cernua*, D: *L. minima*, E: *L. ventricosa*, F: *L. youngii*.

temperature for a few days. Then, they were macerated in the 1:1 mixture of 45% acetic acid and 1N hydrochloric acid at ca 60℃ for 1.5 min, and squashed with 2% aceto-orcein solution. After the observation of their meristematic tissues, the mitotic metaphase chromosomes were characterized by centromeric position followed Lavan *et al.* (1964). The karyotype formulas were based on the positions of centromeres and chromosome length according to Tanaka (1980).

Results and Discussion

Flowers of the material plants are shown in Figure 1. The section followed Duncan (2012), and chromosome numbers (2n) observed in this study and previous counts are described in Table 1. Chromosome numbers of 2n=14, 18 and 28 were counted in this study (Table 1). Among the six species, *L. angelica* was observed karyomorphologically and revealed to have 2n=14 chromosomes for the first time. Chromosome numbers of *L. cernua* (2n=28), *L. minima* (2n=28) and *L. ventricosa* (2n=14) were reconfirmed the previous data described by Spies *et al.* (2008). Present count of 2n=18 in *L. attenuata* and *L. youngii* were different from the previous report (Spies *et al.* 2008, 2009).

The mitotic metaphase and the karyotype are shown in Figure 2 and Figure 3, respectively. Karyotypes of six species studied are described in Table 2.

Lachenalia angelica showed bi-modal karyotype in length which had two relatively long chromosomes and 12 relatively short chromosomes. The two long chromosomes were subtelocentric (st) and short 12 chromosomes were submetacentric (sm) or metacentric (m), then it showed symmetric karyotype in arm ratio. It was suggested that this species was diploid and the basic chromosome number was x=7, because its paired chromosomes showed high similarity on their length and arm ratio.

L. attenuata showed mono-modal karyotype showing gradual decrease in length. All chromosomes were "sm" or "m", and showed symmetric karyotype in arm ratio. It was suggested that this species was diploid and the basic chromosome number was x=9, because its paired chromosomes showed high similarity on their length and arm ratio.

L. cernua showed mono-modal karyotype showing gradual decrease in length. Satellite was observed on the short arm of one chromosome (No.3). The chromosome set consisted of one "st" chromosome (No.7), 11 "sm" and 16 "m" chromosomes, then it showed symmetric karyotype in arm ratio. It was not be able to decide that this species was diploid or tetraploid in this result. And the basic chromosome number was suggested x=14 or 7.

L. minima showed mono-modal karyotype showing gradual decrease in length. All chromosomes were "sm" or "m", and then it showed symmetric karyotype in arm ratio. It was suggested that this species was diploid and the basic chromosome number was x=9, because its paired chromosomes showed high similarity on their length and arm ratio.

L. ventricosa showed mono-modal karyotype showed gradual decrease in length. One chromosome was "st", 11 chromosomes were "sm" and two chromosomes were "m", then showed symmetric karyotype in arm ratio. It was suggested that this species was diploid and the basic chromosome number was x=7, because its paired chromosomes showed high similarity on their length and arm ratio.

L. youngii showed mono-modal karyotype showed gradual decrease in length. All chromosomes were "sm" or "m", and showed symmetric karyotype in arm ratio. It was suggested that this species was diploid and the basic chromosome number was x=9, because its paired chromosomes showed high similarity on their length and arm ratio.

In the genus *Lachenalia*, basic chromosome number of x=5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 21,

Table 2. The results of karyomorphological studies of *Lachenalia*

Species	Information of mitotic metaphase			Polyploidy	Basic chromosome number (x)
	Chromosome number (2n)	Expression from arm ratio*	Expression from chromosome length*		
Lachenalia angelica W. F. Barker ex G. D. Duncan	14	symmetric (6m+6sm+2st)	bi-modal	2x	7
Lachenalia attenuata W. F. Barker ex G. D. Duncan	18	symmetric (10m+8sm)	mono-modal (gradual)	2x	9
Lachenalia cernua G. D. Duncan	28	symmetric (16m+11sm+1st)	mono-modal (gradual)	2x or 4x	14 or 7
Lachenalia minima W. F. Barker	18	symmetric (12m+6sm)	mono-modal (gradual)	2x	9
Lachenalia ventricosa Schltr. ex W. F. Barker	14	symmetric (2m+11sm+1st)	mono-modal (gradual)	2x	7
Lachenalia youngii Baker	18	symmetric (13m+5sm)	mono-modal (gradual)	2x	9

* The mitotic metaphase chromosomes were characterized by centromeric position followed Levan *et al.* (1964). Symbols show regions of centromeres. m: median region, sm: submedian region, st: subterminal region. The karyotype formulas based on the positions of centromeres and chromosome length according to Tanaka (1980).

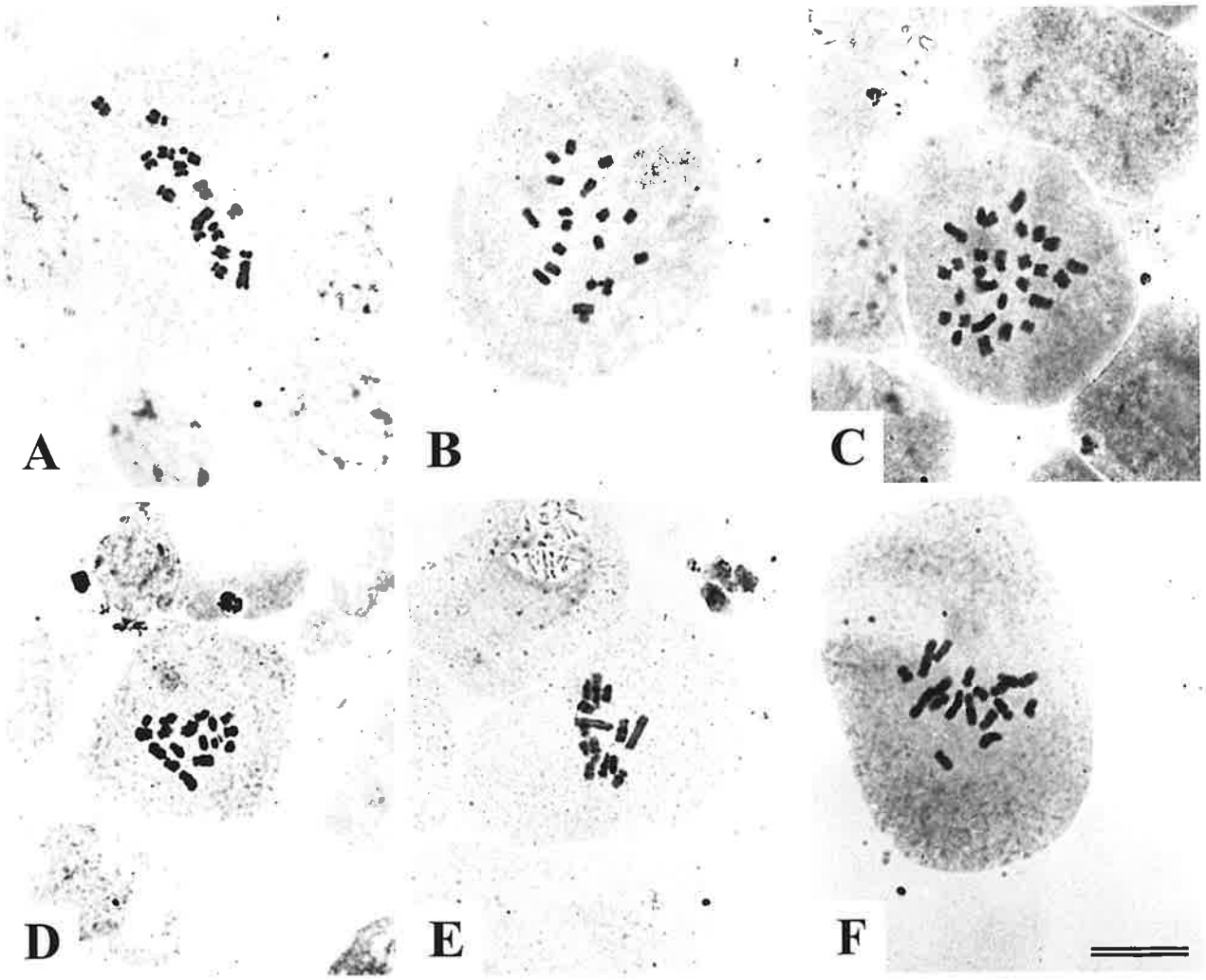

Fig. 2. Somatic chromosomes at mitotic metaphase in *Lachenalia* studied. A: *L. angelica,* B: *L. attenuata*, C: *L. cernua*, D: *L. minima*, E: *L. ventricosa*, F: *L. youngii*. Bar = 10μm.

22 and 28 were already shown, and it was suggested that primary basic chromosome numbers were x=7, 8, 9 and 11 (Spies *et al.* 2011, Kleynhans *et al.* 2012).

Authors already observed 48 taxa (including 41 species, four varieties and three cultivars) of *Lachenalia* and confirmed their chromosome numbers of 2n=14, 15, 16, 17, 18, 22, 23, 24, 26, 28, 42 (Hamatani 2011, Hamatani *et al.* 2012). It was shown by the karyomorphological observation using aceto-orcein staining and molecular cytogenetical observations using 4-6-diamidino-2-phenylindole (DAPI) staining and fluorescent *in situ* hybridization (FISH) with 5S and 18S rDNA probes; that 19 taxa of 2n=14 and one taxon of 2n=15 were x=7 (2x or 2x+1), ten taxa of 2n=16 and two taxa of 2n=17 were x=8 (2x or 2x+1), one taxon of 2n=18 was x=9 (2x), three taxa of 2n=22, one taxon of 2n=23 and one taxon of 2n=24 were x=11 (2x, 2x+1 or 2x+2), two taxa of 2n=24 were x=12 (2x), two taxa of 2n=26 were x=13 (2x), two taxa of 2n=28 were x=14 (2x), three taxa of 2n=28 were x=7 (4x) and one taxon of 2n=42 was x=7 (6x) (Hamatani 2011, Hamatani *et al.* 2012). It was often shown aneuploids and polyploids on the observation for the chromosome of *Lachenalia*. Spies *et al.* (2011) and Kleynhans *et al.* (2012) showed various basic chromosome numbers (x=5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 21, 22 and 28), but this variety of basic chromosome numbers might be clearer by more detail karyomorphological and molecular cytogenetical observations.

Hamatani (2011) reported that ten species which had basic chromosome number of x=8 made one clear clade by the molecular phylogenetical analysis, and they showed strong similarity to each other in karyomorphology and molecular cytogenetics with DAPI staining and FISH method. Also it was suggested that the molecular cytogenetical observation was valid to clarify similarity among 14 species with x=7 chromosomes which were hardy elucidated karyomorphologically, although made a large and uncertain clade by the molecular phylogenetical analysis. In this study, we confirmed two species of *Lachenalia* (*L. angelica*, *L. ventricosa*) having basic chromosome number of x=7. After this, we wish to obtain detail information by molecular cytogenetic methods.

On the other hand, the relationships among the species with x=9, 11, 12 or 14 were not enough examined because the number of observed samples were few (Hamatani 2011, Hamatani *et al.* 2012). In this study, we confirmed three species (*L. attenuata*, *L. minima* and *L. youngii*) with x=9. And, *L. cernua* with 2n=28 was not decided its ploidy and it was leaved a problem.

Therefore, the species with 2n=14, 2n=18, 2n=28, have to been valuable for the future studies on the relationship and speciation of whole of genus *Lachenalia*.

Acknowledgements

We thank Mr. Graham Duncan, Curator of the Bulbous Plants Living Collection and a specialist horticulturist at the South African Biodiversity Institute, Kirstenbosch Botanical gardens for identifying species of *Lachenalia*, and Dr. Tetsuya Sera, Director of the Hiroshima Botanical Garden for his advices to the chromosome analysis.

摘 要

ラケナリア属 (*Lachenalia*) 6種について核形態学的観察を行った. 染色体数は2n=14, 18, 28が観察された. *L. angelica* の 2n=14 は初の報告で, *L. attenuata* の 2n=18, *L. youngii* の 2n=18 は過去の報告と異なる染色体数であった.

Fig. 3. Karyotypes in the six species of *Lachenalia* studied. A: *L. angelica*, B: *L. attenuata*, C: *L. cernua*, D: *L. minima*, E: *L. ventricosa*, F: *L. youngii*. Arrow shows satellite. Bars indicate 5 μm.

Literature Cited

Crosby, T. S. 1986. The genus *Lachenalia*. The Plantsman 8: 129–166.

Duncan, D. 2012. The Genus Lachenalia. Kew Publishing. UK. pp.479.

Fernandes, A. and Neves, J. B. 1962. Sur la caryologie de quelques Monocotyledones africaines. Compt. Rend. De la IV–e Reunion Pleniere de l' Assoc. pour l' Etude Taxinomique de la Flore d' Afrique Tropicale. Lisboa: 439–463.

Hamatani, S. 2011. Characterization of *Lachenalia*, the Liliaceae based on karyomorphological, molecular phylogenetical and molecular cytogenetical studies. Bulletin of The Hiroshima Botanical Garden 29: 1–90.

Hamatani, S., Hashimoto, K. and Kondo, K. 1998. A comparison of somatic chromosomes at metaphase in *Lachenalia* (Liliaceae). Chromosome Science 2: 21–25.

Hamatani, S., Ishida, G., Hashimoto, K. and Kondo, K. 2004. A chromosome study of ten species of *Lachenalia* (Liliaceae). Chromosome Science 8: 55–61.

Hamatani, S., Kondo, K., Kodaira, E. and Ogawa, H. 2007. Chromosome morphology of 12 species and one variety of *Lachenalia* and five species of closely related, allied genera (Liliaceae). Chromosome Botany 2: 79–86.

Hamatani, S., Tagashira, N., Ishida, G. and Kondo, K. 2009. Chromosome relationships among 13 species and one variety of *Lachenalia* (Liliaceae) with the chromosome numbers of 2n=14 and 16 detected by FISH using 5S rDNA and 18S rDNA probes and DAPI staining. Chromosome Botany 4: 57–63.

Hamatani, S, Tagashira, N. and Kondo, K. 2010. Molecular cytogenetic analysis in seven species of *Lachenalia* (Liliaceae) with the chromosome numbers of 2n=18, 22, 23, 26 and 28 by DAPI staining and FISH using 5S rDNA and 18S rDNA probes. Chromosome Botany 4: 57–63.

Hamatani, S., Masuda, Y., Uchida, M., Tagashira, N, Kusaba, M. and Kondo, K. 2012. Molecular cytogenetical and phylogenetical studies of *Lachenalia congesta* (Asparagaceae). Chromosome Botany 7: 47–52.

Johnson, M. A. T. and Brandham, P. E. 1997. New chromosome numbers in petaloid monocotyledons and in other miscellaneous angiosperms. Kew Bull. 52(1): 121–138.

Kleynhans, R., Spies, P. and Spies, J. J. 2012. Cytogenetic and phylogenetic review of the genus *Lachenalia.* Floriculture and Ornamental Biotechnology 6: 98–115.

Levan, A., Fredga, C. and Sandberg, A. A. 1964. Nomenclature of centromeric position on chromosomes. Hereditas 52: 201–220.

Moffett, A. A. 1936. The cytology of *Lachenalia.* Cytologia 7: 490–498.

Spies, P. 2004. Phylogenetic relationships of the genus *Lachenalia* with other related liliaceous taxa. MSc., Univ. of the Free State. 155pp.

Spies, J. J., du Preez, J. L., Minnaar, A. and Kleynhans, R. 2000. Hyacinthaceae: Chromosome studies of African plants. 13. *Lachenalia mutabilis*, *L. pustulata* and *L. unicolor*. Bothalia 30: 106–110.

Spies, J. J., van Rooyen, P. and Kleynhans, R. 2002. The subgeneric delimitation of *Lachenalia* (Hyacinthaceae). Acta Horticulturae 570: 225–232.

Spies, J. J., Spies, P., Reinecke, S. M., Kleynhans, R., Duncan, G. D. & Edwards, T. J. 2008. *Lachenalia*. In: K. Marhold (ed.) , IAPT/IOPB chromosome data 5, Taxon 57(2): 554–555.

Spies, J. J., Spies, P., Reinecke, S. M. C., Minnaar, A., du Preez, J. L. and Kleynhans, R. 2009. *Lachenalia*. In: K. Marhold (ed.), IAPT/IOPB chromosome data 8. Taxon 58 (4): 1288–1289.

Spies, J. J., Spies, P. and Kleynhans, R. 2011. Basic chromosome numbers in the genus *Lachenalia* (Hyacinthaceae). Philos. Trans. Genet. 1: 67–79.

Tanaka, R. 1980. The karyotype. *In* Kihara, H. (ed.), Plant genetics 1, pp. 335–358. Shokabo Co., Tokyo. (In Japanese).

de Wet, J. M. J. 1957. Chromosome numbers in the Scilleae. Cytologya 22: 145–159.

Appendix : Measurements of somatic chromosomes of *Lachenalia* at mitotic metaphase

Table 3. *L. angelica*, 2n = 14

Chromosome	Length (μm)	Relative length (%)	Arm ratio	Form
1	1.01+3.25=4.26	12.24	3.22	st
2	1.01+3.19=4.20	12.07	3.16	st
3	1.05+1.83=2.88	8.27	1.74	sm
4	0.88+1.79=2.67	7.67	2.03	sm
5	1.09+1.50=2.59	7.44	1.38	m
6	1.07+1.30=2.37	6.81	1.21	m
7	0.78+1.46=2.24	6.43	1.87	sm
8	0.76+1.40=2.16	6.21	1.84	sm
9	0.91+1.21=2.12	6.09	1.33	m
10	0.88+1.21=2.09	6.00	1.38	m
11	0.84+1.17=2.01	5.77	1.39	m
12	0.76+1.13=1.89	5.43	1.49	m
13	0.66+1.17=1.83	5.26	1.77	sm
14	0.43+1.07=1.50	4.31	2.49	sm

Table 4. *L. attenuata*, 2n = 18

Chromosome	Length (μm)	Relative length (%)	Arm ratio	Form
1	0.85+1.81=2.66	7.38	2.13	sm
2	0.91+1.72=2.63	7.30	1.89	sm
3	0.82+1.66=2.48	6.88	2.02	sm
4	0.91+1.40=2.31	6.41	1.54	m
5	0.67+1.60=2.27	6.30	2.39	sm
6	0.70+1.49=2.19	6.07	2.13	sm
7	0.85+1.29=2.14	5.94	1.52	m
8	0.51+1.43=1.94	5.38	2.80	sm
9	0.78+1.15=1.93	5.35	1.47	m
10	0.67+1.16=1.83	5.08	1.73	sm
11	0.79+1.02=1.81	5.02	1.29	m
12	0.77+1.04=1.81	5.02	1.35	m
13	0.74+1.05=1.79	4.97	1.42	m
14	0.76+1.01=1.77	4.91	1.33	m
15	0.79+0.90=1.69	4.69	1.14	m
16	0.60+1.05=1.65	4.58	1.75	sm
17	0.70+0.90=1.60	4.44	1.29	m
18	0.62+0.93=1.55	4.30	1.50	m

Table 5. *L. cernua*, 2n = 28

Chromosome	Length (μm)	Relative length (%)	Arm ratio	Form
1	1.21+2.03=3.24	6.00	1.68	m
2	1.05+2.12=3.17	5.87	2.02	sm
3	0.39+0.81+1.46=2.66	4.93	1.22	m
4	0.89+1.70=2.59	4.80	1.91	sm
5	0.81+1.75=2.56	4.74	2.16	sm
6	0.66+1.85=2.51	4.65	2.80	sm
7	0.60+1.88=2.48	4.59	3.13	st
8	0.75+1.60=2.35	4.35	2.13	sm
9	0.74+1.25=1.99	3.69	1.69	m
10	0.87+1.10=1.97	3.65	1.26	m
11	0.57+1.28=1.85	3.43	2.25	sm
12	0.66+1.16=1.82	3.37	1.76	sm
13	0.69+1.11=1.80	3.33	1.61	m
14	0.71+1.01=1.72	3.19	1.42	m
15	0.44+1.27=1.71	3.17	2.89	sm
16	0.54+1.11=1.65	3.06	2.06	sm
17	0.75+0.89=1.64	3.04	1.19	m
18	0.59+1.04=1.63	3.02	1.76	sm
19	0.60+0.99=1.59	2.95	1.65	m
20	0.45+1.10=1.55	2.87	2.44	sm
21	0.63+0.90=1.53	2.83	1.43	m
22	0.57+0.93=1.50	2.78	1.63	m
23	0.62+0.86=1.48	2.74	1.39	m
24	0.59+0.86=1.45	2.69	1.46	m
25	0.63+0.77=1.40	2.59	1.22	m
26	0.60+0.80=1.40	2.59	1.33	m
27	0.62+0.75=1.37	2.54	1.21	m
28	0.57+0.80=1.37	2.54	1.40	m

Table 6. *L.minima*, 2n = 18

Chromosome	Length(μm)	Relative length	Arm ratio	Form
1	0.70+1.98=2.68	7.89	2.83	sm
2	0.88+1.66=2.54	7.48	1.89	sm
3	1.00+1.18=2.18	6.42	1.18	m
4	0.96+1.12=2.08	6.12	1.17	m
5	0.73+1.26=1.99	5.86	1.73	sm
6	0.80+1.16=1.96	5.77	1.45	m
7	0.93+1.02=1.95	5.74	1.10	m
8	0.76+1.10=1.86	5.48	1.45	m
9	0.76+1.09=1.85	5.45	1.43	m
10	0.73+1.12=1.85	5.45	1.53	m
11	0.77+1.06=1.83	5.39	1.38	m
12	0.55+1.26=1.81	5.33	2.29	sm
13	0.80+0.99=1.79	5.27	1.24	m
14	0.66+1.02=1.68	4.95	1.55	m
15	0.66+0.99=1.65	4.86	1.50	m
16	0.47+1.03=1.50	4.42	2.19	sm
17	0.47+0.99=1.46	4.30	2.11	sm
18	0.57+0.73=1.30	3.83	1.28	m

Table 7. *L.ventricosa*, 2n = 14

Chromosome	Length(μm)	Relative length (%)	Arm ratio	Form
1	0.77+3.88=4.65	12.30	5.04	st
2	1.17+3.18=4.35	11.50	2.72	sm
3	0.91+2.59=3.50	9.25	2.85	sm
4	1.08+2.11=3.19	8.43	1.95	sm
5	0.81+1.90=2.71	7.17	2.35	sm
6	0.73+1.96=2.69	7.11	2.68	sm
7	0.85+1.63=2.48	6.56	1.92	sm
8	0.77+1.63=2.40	6.35	2.12	sm
9	0.91+1.34=2.25	5.95	1.47	m
10	0.90+1.16=2.06	5.45	1.29	m
11	0.72+1.28=2.00	5.29	1.78	sm
12	0.64+1.27=1.91	5.05	1.98	sm
13	0.58+1.28=1.86	4.92	2.21	sm
14	0.64+1.13=1.77	4.68	1.77	sm

Table 8. *L.youngii*, 2n = 18

Chromosome	Length(μm)	Relative length	Arm ratio	Form
1	1.47+2.59=4.06	8.42	1.76	sm
2	1.38+2.61=3.99	8.28	1.89	sm
3	1.38+2.05=3.43	7.12	1.49	m
4	1.40+1.97=3.37	7.00	1.41	m
5	0.98+1.97=2.95	6.12	2.01	sm
6	1.05+1.60=2.65	5.50	1.52	m
7	1.05+1.60=2.65	5.50	1.52	m
8	0.98+1.67=2.65	5.50	1.70	sm
9	0.99+1.60=2.59	5.37	1.62	m
10	0.99+1.60=2.59	5.37	1.62	m
11	0.86+1.44=2.30	4.77	1.67	m
12	0.72+1.59=2.30	4.77	2.24	sm
13	0.86+1.43=2.29	4.75	1.66	m
14	0.82+1.34=2.16	4.48	1.63	m
15	0.92+1.24=2.16	4.48	1.35	m
16	1.02+1.12=2.14	4.44	1.10	m
17	0.86+1.14=2.00	4.15	1.33	m
18	0.86+1.05=1.91	3.96	1.22	m