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Morphological multivariate analyses of *Isodon excisus* complex (Lamiaceae) in Korea

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ABSTRACT: The taxonomy of the *Isodon excisus* complex has been ambiguous and problematic because the morphological characters, especially characters related to the leaf distinguishing subgroups of the complex in the original descriptions, are variable. To elucidate the taxonomic structure of the *I. excisus* complex in Korea, 34 characters were measured from 70 OTUs representing different locations and analyzed by principal component analysis (PCA). The analysis showed that principle component axis 1, 2, 3 (PC1, PC2, PC3) represents 52.0% of the total variance and characters showing high loading values for PC1 were leaf shape, density of non-glandular hairs on the lower surface of the leaf, and characters related to the teeth of the leaf. The length of apical tooth and the angle between two widest points of the leaf were highly correlated to PC2 and PC3, respectively. Three-dimensional scatter plotting of OTUs for PC1, PC2, and PC3 axis showed that the areas of previously recognized three subgroups *of I. excisus* completely overlapped. Our result supported that just one taxon, *I. excisus* var. *excisus,* should be recognized in the complex at the variety level.

Keywords: *Isodon excisus* complex, PCA, multivariate analysis, Lamiaceae, *Isodon*

A recent classification of Lamiaceae by Harley et al. (2004) contains seven subfamilies: Symphorematoideae, Viticoideae, Ajugoideae, Prostantheroideae, Scutellarioideae, Lamioideae, and Nepetoideae. In this system, *Isodon* (Schrad. ex Benth) Spach is included in the subfamily Nepetoideae. *Isodon* contains about 100 species distributed in tropical and subtropical regions in Asia for most species, and in Africa for a few species (Mabberley., 1987; Li, 1988; Judd et al., 2008; Maki et al., 2010; Zhong et al., 2010). Although there was some controversy over the genus boundary, recent taxonomic review of the genus concluded that it clearly distinguished from related genera, *Siphocranion* Kudo, *Skapanthus* C. Y. Wu & H. W. Li, and *Plectranthus* L'Hér., based on morphological characters such as the adnation degrees of stamens and petals, and connation degrees of petals and sepals (Li, 1988). *Isodon* have flowers with stamens inserted on the lower part of the corolla but *Siponcranion* have flowers with stamens inserted near the throat of the corolla. Two-lipped calyx in *Plectranthus* is distinguished from five equal or subequal teethed *Isodon*. Posterior filaments of *Skapanthus* are dilated outside the base compared to those of *Isodon*. However, some authors still consider these four genera as a genus, *Plectranthus* (Li, 1988).

Recent bibliographic works for Korean flora (Korea National Arboretum and the Plant Taxonomic Society of Korea, 2007) have reported that there are four species, six varieties, and one forma in the genus *Isodon* in Korea: *I. excisus* (Maxim.) Kudo, *I. excisus* var. *chiisanensis* (Nakai) Lee, *I. excisus* var. *coreanus* (Nakai) Lee, *I. excisus* for. a*lbiflorus* (Sakata) Hara, *I. inflexus* (Thunb.) Kudo, *I. inflexus* var. *macrophyllus* (Maxim.) Kudo, *I. inflexus* var. *transiticus* (Matsum. et Kudo) Kudo, *I. inflexus* var. *microphyllus* (Nakai) Kudo, *I. inflexus* var. *canescens* (Nakai) Kudo, *I. japonicas* (Burmann) Hara, and *I. serra* (Maxim.) Kudo. However, subgroups in *I. excisus*, so called *I. excisus* complex, are taxonomically confusing and need to be reviewed because of their variable characters.

Since *Plectranthus excisus* Maxim (1859) have been reported, Nakai (1911) accepted this genus name. However, Mori (1921), Kudo (1929), and Hara (1972) placed this taxon in *Plectranthus*, in *Isodon,* and in *Rabdosia,* respectively. Whatever the genus name is, three taxonomic groups were continuously recognized in the species by most authors: var. *excisus*, var. *chiisanensis*, and var. *coreanus* (Mori, 1921; Nakai, 1934; Lee, 1985; Y. N. Lee, 1996; Korean National Arboretum and the Plant Taxonomic Society of Korea, 2007). However, nomenclatural problems of *I. excisus* var. *chiisanensis* (Nakai) T. B. Lee and *I. excisus* var. *coreanus* (Nakai) T. B. Lee have been reported in the recent bibliographic study (Kim et al., 2005). After Kudo (1929) recognize

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Plectranthus excisus as a species of *Isodon*, two varieties of *P. excisus* had been transferred to *Isodon* without indication of basionyms making them invalid names (Kim et al., 2005). Once these are recognized as invalid names, it is not recommended to use these names in the research paper. Therefore, in this paper, we used "CHIISANENSIS and COREANUS" instead of *I. excisus* var. *chiisanensis* and var. *coreanus*.

In the original description, *Plectranthus excisus* var. *chiisanensis* and var. *coreanus* were distinguished from the original variety, var. *excisus,* by wide leaf blade (width is longer than length) and truncated leaf base and by round leaf shape and shorter and wider leaf teeth, respectively (Nakai, 1915, 1921). However, continuation of character states has been observed among these taxa. In this study, we called all of these diagnostically ambiugous taxa as *I. excisus* complex.

In this study, we aimed to search for the taxonomic structure (*sensu* Sneath and Sokal, 1973) of *I. excisus* complex in Korea by reinvestigating previously reported diagnostic characters from original descriptions and analyzing the data with a multivariate data analysis approach, principal component analysis (PCA).

Materials and Methods

Materials used in this study included samples collected from 2009 to 2011 and herbarium specimens deposited in the herbaria including Sungshin Women's University (SWU), Sung Kyun Kwan University (SKK), Seoul National University (Dept. of Biology: SNU; Dept. of Forestry: SNUA), Korea University (KSU), Kangwon National University (KWNU), Choongbook University (CBU), National Institute of Biological Sciences in Korea (NIBR), and National Arboretum in Korea (KH). The total specimen number in this study was about 600.

Based on the literature, we recognized three subgroups in the *I. excisus* complex. Although there was some ambiguity because of the continuation of character states among the taxa in the complex, we attempted preliminary identification based on the original descriptions in each taxon (Nakai, 1915, 1921). Measurements were taken from 70 Operational Taxonomic Units (OTUs) selected from fully mature plants from different locations for 34 morphological characters (Table 1, Fig. 1) (Appendix). Based on our preliminary identification, these OTUs represented six COREANUS (*I. excisus* var. *coreanus*), nine CHIISANENSIS (*I. excisus* var. *chiisanensis*), and 55 EXCISUS (*I. excisus* var. *excisus*) samples. We excluded *I. excisus* for. *albiflorus*, which has white flowers, in the PCA because of insufficient specimen number. The characters we measured include diagnostic characters cited in the original descriptions of each taxon in the complex such as the length and width of leaf, the length of apical teeth in the leaf, width and height of teeth in the leaf, and angle of serration in the tooth.

Table 1. Morphological characters of *I. excisus* complex used for principal component analysis

Quantitative characters [unit]
1. Length of the leaf [mm]*
2. Length of the petiole [mm]*
3. Length of the leaf blade [mm]*
4. Width of the leaf blade [mm]*
5. Length of the most broad part to the apex [mm]*
6. 3 / 4
7. Density of glandular hairs on the upper surface of the leaf [no./1 mm^2]*#
8. Density of glandular hairs on the lower surface of the leaf [no./1 mm^2]*#
9. Density of non-grandular hairs on upper surface of the leaf [no./1 mm^2]*#
10. Density of non-grandular hairs on lower surface of the leaf [no./ 1 mm^2]*#
11. Length of the apical tooth [mm]*
12. Width of the apical tooth [mm]*
13. Length between apexes of two first teeth [mm]*
14. Length of the leaf base [mm]*
15. Width of the leaf base [mm]*
16. 3 / 2
17. 3 / 14
18. 3 / 5
19. 14 / 15
20. 3 / 11
21. 4 / 12
22. 13 / 12
23. Angle of the leaf apex [°]*
24. Angle between apexes of two first teeth [°]*
25. Angle of the leaf base [°]*
26. Angle between two widest points the leaf base [°]*
27. Angle between two widest points the leaf [°]*
28. Number of teeth (one half) [no.]*
29. Average width of the 2^{nd}, 3^{rd}, and 4^{th} tooth from the apex [mm]*
30. Average height of the 2^{nd}, 3^{rd}, and 4^{th} tooth from the apex [mm]*
31. Length of the tooth base to the tooth apex (mean value among the 2^{nd}, 3^{rd}, and 4^{th} tooth) [mm]*
32. 31 / 29
33. 31 / 30
34. Angle of the teeth apex (mean value among the 2^{nd}, 3^{rd}, and 4^{th} tooth) [°]*

*a fully mature leaf in each specimen

#a middle part between two longest secondary veins in a fully matured leaf

Numerical Taxonomy and Multivariate Analysis System (NTSYS, ver. 1.7; Rohlf, 1992) was used for PCA. The standardization of raw data was performed using STAND commend. A similarity matrix was generated using the SIMINT command with the CORR option, and canonical vectors were generated using the CVA and PROJ commands. For three-dimensional scatter plotting, SAS/GRAPH (ver 9.1; SAS institute Inc., USA) was used.

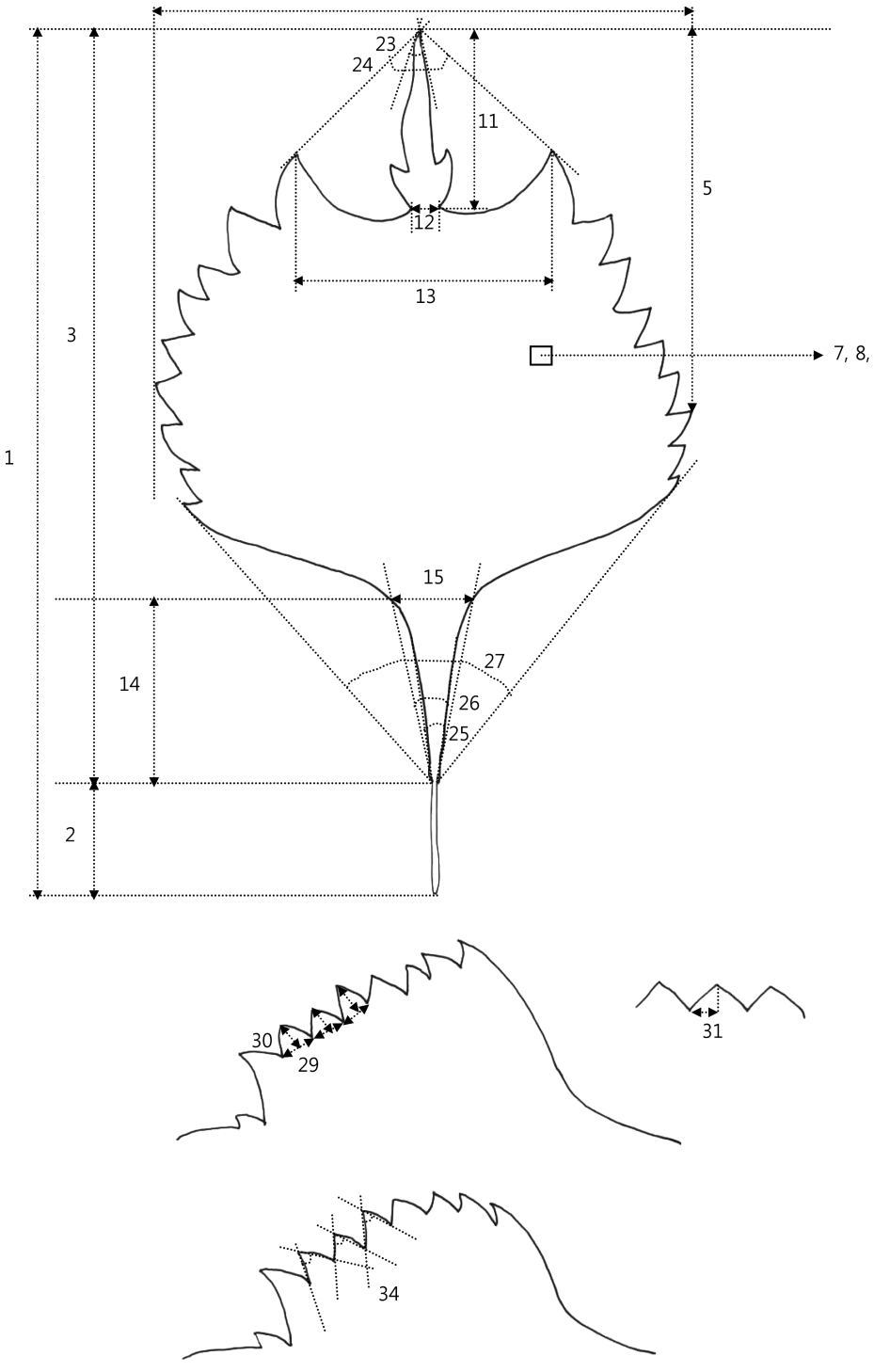

Fig. 1. Representation of characters used for the principal component analysis of *I. excisus* complex.

Results

PCA for 70 OTUs from *I. excisus* complex was performed based on 34 morphological characters (C1~C34; Table 1; Fig. 1). The principal component loading values are listed in Table 2. The first three principal components showed 26.3%, 16.2%, and 9.5% of the total variance, respectively. Cumulative value of variance of the first three principal components was 52.0%

Table 2. Loadings of the 34 morphological characters for the three principle component axes from the analysis of 70 individuals of the *I. excisus* complex in Korea.

Character	PC 1	PC 2	PC 3
C1	−0.54982	0.64355*	0.35875
C2	−0.51491	0.35705	−0.08242
C3	−0.40488	0.54586	0.55857
C4	−0.13673	0.17488	−0.36387
C5	−0.06114	0.53012	0.49322
C6	−0.84291*	−0.38418	0.27552
C7	0.41622	−0.37217	−0.35829
C8	0.41990	0.16045	−0.19190
C9	−0.62914*	−0.39089	−0.20104
C10	−0.87610*	−0.35171	0.02500
C11	0.02238	0.72213*	0.33111
C12	−0.26804	−0.02648	0.21080
C13	−0.12906	0.54114	−0.15859
C14	−0.61266*	0.36601	−0.18092
C15	−0.41133	0.08912	0.09856
C16	0.14898	−0.50457	0.45852
C17	0.09088	−0.53686	0.57380
C18	−0.49557	−0.34638	0.14208
C19	−0.78937*	−0.21458	−0.03763
C20	−0.30686	−0.62300*	−0.17570
C21	0.20551	0.23382	−0.24054
C22	−0.04346	0.38164	−0.23280
C23	0.15325	0.05392	−0.16980
C24	0.11332	−0.41688	−0.69095*
C25	0.54475	−0.31228	0.56006
C26	0.55028	−0.35997	0.47064
C27	0.69921*	−0.32001	−0.09772
C28	0.19899	−0.52693	0.00190
C29	−0.52613	0.00254	−0.06651
C30	−0.70916*	0.24105	−0.05534
C31	−0.86983*	−0.24305	−0.11051
C32	−0.87419*	−0.41010	0.05771
C33	−0.82503*	−0.49028	0.08100
C34	0.38755	−0.44882	0.35560
Eigenvalue	8.94398	5.50479	3.2380
% of variance	26.30%	16.20%	9.50%
Cumulative % of variance	26.30%	42.50%	52.00%

(Table 2). In the first principal component (PC1), important characters, of which loading values were over ± 0.8, were ratio of leaf blade width to leaf blade length (C6), density of non-glandular hairs on the lower surface of the leaf (C10), characters related to the teeth of the leaf (C31, C32, C33). Characters showing relatively high vales (over ± 0.6) were density of non-glandular hair on the lower surface of the leaf (C9), length of the leaf base (C14), ration between length and width of the leaf base (C19), angle between two widest points in the leaf (C27), and height of the teeth (C30). The length of the apical tooth (C11) was the highest loading character on PC2 (0.72) and values over ± 0.6 were also found in the length of the leaf (C1) and ratio between length of the apical tooth and length of the leaf blade (C20). In PC3, PC loading values were relatively low compared to PC1 and PC2. The highest PC loading values were found in C24 (angle between two widest points of the leaf) for PC3.

The OTUs were plotted in the three-dimensional diagram using the first three principle components (Fig. 2). The scatter plot showed that OTUs representing three subgroups in the complex were difficult to distinguish each other. OTUs of COREANUS (cubes) were placed at the center of PC1 × PC2 plane. OTUs of EXCISUS had relatively low PC1 values and high PC2 values compared to CHIISANENSIS. However, most areas of these three groups of OTUs overlapped representing all OTUs used in PCA should be recognized as one taxon.

Discussion

PCR analysis showed that there were no distinguished morphological subgroups in the *I. excisus* complex. This result does not support the recognition of two subgroups in the complex, CHIISANENSIS and COREANUS that were previously recognized as *Plectranthus* var. *chiisanensis* and *P.* var. *coreanus*. As Korean authors, Chung (1957) and W.T. Lee (1996) included *I. excisus* in their manual for the Korean flora but did not recognize any varieties in the species. Our analysis agrees with their point of view for the infraspecific taxa in *I. excisus*.

In most previous reports, var. *chiisanensis* is called "Deunggun-Oribangpul (round leafed *I. excisus*)" and var. *coreanus* is called "Chiri-Oribangpul (*I. excisus* located in Mt. Chiri)". However, actually var. *chiisanensis* was first found in Mt. Chiri and leaves of var. *coreanus* were of the round type in the original descriptions of these taxa. It seems that the Korean name for var. *chiisanensis* and var. *coreanus* have been erroneously used in most Korean plant manuals (Lee, 1985; Y.N. Lee, 1996; Korean National Arboretum and the Plant Taxonomic Society of Korea, 2007). Furthermore, Kim et al. (2005) have already pointed out that *I. excisus* var. *chiisanensis* (Nakai) T. B. Lee and *I. excisus* var. *coreanus* (Nakai) T. B. Lee are invalid names because no basionyms

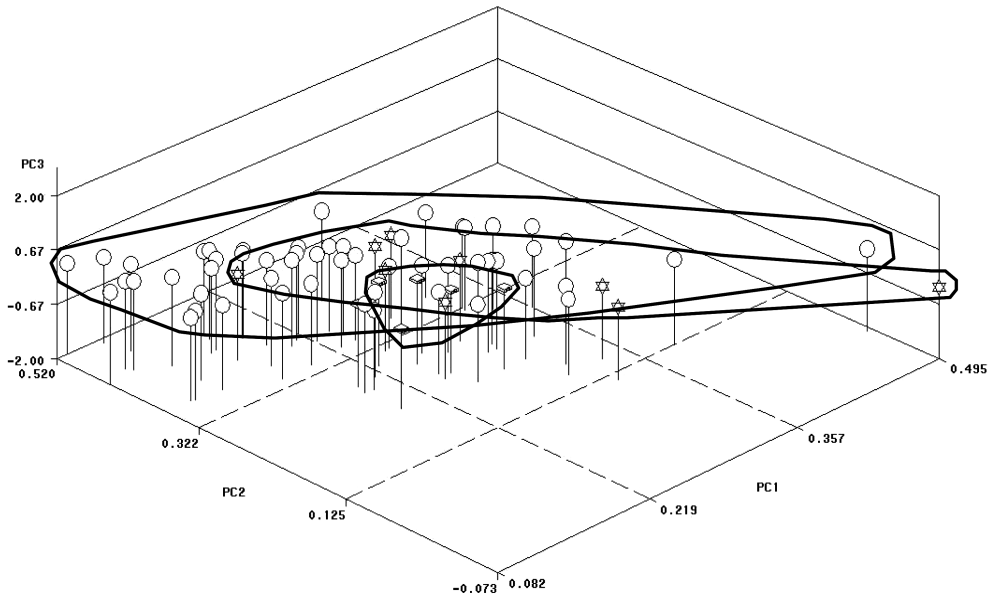

Fig. 2. Three-dimensional scatter plotting of OTUs representing *I. excisus* complex. Icons indicate pre-identification of OTUs before the PCA based on the morphological characters indicated in the original descriptions. Balloons, EXCISUS; cubes, COREANUS; stars, CHIISANENSIS.

had been cited in the combinations. However these arguments are now meaningless because the current study does not recognize any distinguished subgroups in the complex except *I. excisus* for. *albiflorus*, which is a albino from of *I. excisus*.

In China, *I. excisus* is distributed in Hebei, Heilongjiang, Jilin, Liaoning, and Shangxi but a recent study does not recognize any varieties in the species (Li and Hedge, 1994). In Japan, *I. excisus* var. *hakusanensis* Kudo have been reported (Kudo, 1929). However, currently this taxon is treated as *I. umbrosus* var. *hakusanensis* (Kudo) K. Asano (Iwatsuki et al., 1993). Based on the literature, OTUs we included in the analysis represented varieties in the species well. However, to clarify taxonomic structure of the complex, our further study needs to include *I. excisus* in China and Japan.

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APPENDIX. List of samples used for PCA.

Gangwon-do: Daegwanryeong Daegwanryeong-myeon Pyeongchang-gun, Aug. 20-21 1969, *T. Lee s. n.* (SNUA); Dakmongnyeong Wangsan-myeon Gangneung-si, Jul. 27 2007, *H.-G. Oh et al. Daegwallyeong-0087* (KH); Dong River Yeongwol-eup Yeongwol-gun, Sep. 3 2005, *Hong et al. KHUS0312* (KH); Duwibong Jeongseon-gun, Jul. 3 2008, *S.-H. Oh et al. s. n.* (KH); Gwangdeok-ri Sanae-myeon Hwacheon-gun, Sep. 30 2006, *E.-S. Jeon ESJeon1777* (KH); Mt. Baegun Panbu-myeon Wonju-si, Aug. 17 2003, *K.-J. Kim et al. s. n.* (KSU); Mt. Baekjeokm Pyeongchang-gun, Sep. 5 2008, *J.-C. Yang Baekjeoksan80905-9* (KH); Mt. Cheolgapryeong Yeonok-myeon Gangneung-si, Jul. 24 1998, *H.-W. Lee et al. s. n.* (SNU); Mt. Chiak Socho-myeon Wonju-si, Sep. 21 1996, *E. Cho s. n.* (SNU); Mt. Daeam Seoheung-ri Seohwa-myeon Inje-gun, Sep. 19 2008, *W. Paik NAPI-0349, NAPI-0265* (KH); Mt. Deokhang Singi-myeon Samcheok-si, Aug. 6 2005, *S. Lee et al. s. n.* (KH); Mt. Duta Samcheok-si, Sep. 8 2007, *J. Hyun et al. s. n.* (KH); Mt. Gari Duchon-myeon Hongcheon-gun, Sep. 18 1976, *Y. Kil s. n.* (KWNU); Mt. Gyebang Jinbu-myeon Pyeongchang-gun, Aug. 23 2005, *K.-J. Kim et al. s. n.* (KSU) two sheets; Mt. Gyebang Jinbu-myeon Pyeongchang-gun, Jul. 26 2003, *K. Heo et al. s. n.* (SKK); Mt. Jeombong Seorak-dong Sokcho-si, Jul. 9 2008, *W. Paik NAPI-0257* (KH); Mt. Ma Toseong-myeon Goseong-gun, Sep. 20 2008, *W. Paik NAPI-0331* (KH); Mt. Maebong Yongdae-ri Buk-myeon Inje-gun, Sep. 5-6 2008, *H.-W. Kim et al. s. n.* (KSU); Mt. Myeongseong Galmal-eup Cheorwon-gun, Oct. 16 2004, *S.-H. Park s. n.* (KH); Mt. Odae Hongcheon-gun, Aug. 9 2000, *Y. Choi et al. s. n.* (KH); Mt. Odae Jinbu-myeon Pyeongchang-gun, Aug. 2 1972, *W. Lee s. n.* (KWNU); Mt. Samyeong Yanggu-eup Yanggu-gun, Jul. 17 2008, *W. Paik NAPI-0340, NAPI-0338* (KH); Mt. Seorak Seorak-dong Sokcho-si, Oct. 9 2001, *S.-H. Park et al. s. n.* (KH); Mt. Seorak Seorak-dong Sokcho-si, Sep. 21 1979, *G. Lee s. n.* (SNU); Mt. Taebaek Sodo-dong Taebaek-si, Jul. 19 2007, *Kim et al. s. n.* (KH); Mt. Yonghwa Chuncheon-si, Sep. 27 2007, *W. Lee et al. s. n.* (KWNU); Mt. Yonghwa Hwacheon-gun, Sep. 5 2008, *H.-J. LeeYonghwasan80905-1* (KH); Mt.Bangtae Inje-gun, Aug. 16 2008, *W. Paik NAPI-0364* (KH); Samhyeongjebong Seorak-dong Sokcho-si, Aug. 1 2008, *W. Paik NAPI-0372* (KH). **Gyeonggi-do:** Mt. Baegun Idong-myeon Pocheon-si, Sep. 4 2004, *M.-J. Song et al. s. n.* (KSU); Mt. Chungnyeong Sudong-myeon Namyangju-si, Oct. 10 2009, *Y. Ma 2010065* (SWU); Mt. Chungnyeong Sudong-myeon Namyangju-si, Sep. 13 2002, *Hong et al. s. n.* (KH); Mt. Hwaak Hwaak-ri Buk-myeon Gapyeong-gun, Aug. 1 2006, *K.-J. Kim et al. s. n.* (KSU); Mt. Hwaak Hwaak-ri Buk-myeon Gapyeong-gun, Sep. 23 2006, *S. Lee et al. s. n.* (NIBR); Mt. Hwaak Hwaak-ri Buk-myeon Gapyeong-gun, Sep. 9 2005, *Y.-D. Kim s. n.* (KSU); Mt. Jugyeop Sohol-eup Pocheon-si, Sep. 5 2008, *S.-H. Oh Jugyeopsan80905-106* (KH); Mt. Myeongji Jeongmok-ri Buk-myeon Gapyeong-gun, Aug. 1 2006, *H.-T. Kim et al. s. n.* (KSU); Mt. Myeongji Jeongmok-ri Buk-myeon Gapyeong-gun, Sep. 9 1995, *H. Roh281* (SNU); Mt. Jiri Hamyang-gun, Aug. 25 2004, *B. Oh et al. s. n.* (CBU); Mt. Jiri Hamyang-gun, Oct. 10 2004, *B. Oh et al. s. n.* (CBU); Mt. Jiri Hamyang-gun, Sep. 2 2008, *Y.-K. Kim et al. s. n.* (KSU); Mt. Jiri Sancheong-gun, Summer 1997, *K.-J. Kim et al. s. n.* (KSU). **Gyeongsangbuk-do:** Daehyeon-ri Seokpo-myeon Bonghwa-gun, Jul. 12 2008, *K. Bae et al. BKH-080300* (KH); Mt. Guryong Chunyang-myeon Bonghwa-gun, Jul. 27 2008, *J. Hyun et al. 1001027* (KH). **Jeollanam-do:** Mt. Baegyang Bukha-myeon Jangseong-gun, Oct. 25 2001, *E.-S. Jeon et al. s. n.* (KH); Mt. Jiri Sandong-myeon Gurye-gun, Aug. 17 1976, *W. Lee s. n.* (KWNU); Mt. Jiri Sandong-myeon Gurye-gun, Aug. 18 2004, *B. Oh et al. Guryegun(Jirisan)-040918-028* (KH); Mt. Jiri Sandong-myeon Gurye-gun, Aug. 6 2006, *K.-J. Kim et al. s. n.* (KSU); Mt. Jiri Sandong-myeon Gurye-gun, Jul. 1 1913, *T. Nakai s. n.* (SNUA); Mt. Jiri Sandong-myeon Gurye-gun, Jul. 18 1961, *T. Lee s. n.* (SNUA); Mt. Jiri Sandong-myeon Gurye-gun, Jul. 23 1963, *T. Lee et al. s. n.* (SNUA); Mt. Jiri Sandong-myeon Gurye-gun,

Jul. 9 2006, *Y.-H. Kim et al. s. n.* (KSU); Mt. Jiri Sandong-myeon Gurye-gun, Sep. 18 2004, *B. Oh et al. s. n.* (CBU); Mt. Jiri Sandong-myeon Gurye-gun, Sep. 24 1990, *B. Lee s. n.* (SNU); Mt. Jiri Sandong-myeon Gurye-gun, Sep. 24 1990, *S. Kang s. n.* (SNU); Mt. Jiri Sandong-myeon Gurye-gun, Sep. 9 2006, *J.-S. Kim et al. s. n.* (KSU); Mt. Mudeung Jisan-dong Dong-gu Gwangju, Aug. 11 2000, *H.-T. Im s. n.* (NIBR). **Jeollabuk-do:** Mt. Daedun Unju-myeon Wanju-gun, Oct. 7 2004, *K. Yoo et al. Wanjugun (Daedunsan)-041007-062* (KH); Mt. Deogyu Seolcheon-myeon Muju-gun, Aug. 17 2007, *K. Heo, et al. s. n.* (NIBR); Mt. Deogyu Seolcheon-myeon Muju-gun, Aug. 2 1959, *T. Jung s. n.* (SKK); Mt. Deogyu Seolcheon-myeon Muju-gun, Jul. 31 1987, *S. Lee s. n.* (SKK); Mt. Deogyu Seolcheon-myeon Muju-gun, Sep. 7 2002, *J. Kim et al. 2002-0340* (KH); Mt. Segeol Sannae-myeon Namwon-si, Sep. 10 2005, *Y.-H. Kim et al. s. n.* (KSU); Mt. Seongsu Jangsu-gun, Jul. 3 2004, *W. Paik s. n.* (KH); Mt. Unjang Jinan-gun, Aug. 13 1976, *W. Lee s. n.* (KWNU). **Chungcheongbuk-do:** Mt. Minjuji Sangchon-myeon Yeongdong-gun, Sep. 10 2005, *C.-Y. Yoon YC058* (KH); Mt. Sobaek Danyang-eup Danyang-gun, Aug. 6 1999, *C.-W. Park et al. s. n.* (SNU); Mt. Sokri Boeun-gun, 1 1958, *T. Lee s. n.* (SNUA); Mt. Sokri Boeun-gun, 1 1959, *T. Lee s. n.* (SNUA); Mt. Sokri Boeun-gun, Sep. 15 1966, *T. Lee et al. s. n.* (SNUA); Mt. Worak Jecheon-si, Sep. 22 2001, *G. Park et al. s. n.* (KH).