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Comparative anatomy of the needles of *Abies koreana* and its related species

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Abstract: The morphological traits of the resin duct of the needle leaves of *Abies koreana* E.H.Wilson, an endemic species of Korea, and its related species were analysed. The quantitative traits of the resin ducts from 289 individuals were measured, and the observed resin duct types were compared with previous studies. As a result, each characteristic of the resin duct showed some clinal variations with latitude changes. The variations had extreme values in *A. nephrolepis* (Trautv.) Maxim. on Mt. Sikhote-Alin (Russia) and in *A. koreana* on Mt. Halla (Korea). In addition, it was difficult to suggest just one type of resin duct in some taxa. Thus, further studies on the type of resin duct will need to survey the entire distribution area of a taxon and analyse the quantitative traits to determine the range of morphological variation.

Key words: *Abies*, clinal variation, cross-section, resin duct

1. Introduction

There are about 50 species of firs throughout the world (Farjon, 1990). On the Korean peninsula, there are 3 species (Liu, 1971; Cheng & Fu, 1978): *Abies holophylla* Maxim., *A. koreana* E.H.Wilson, and *A. nephrolepis* (Trautv.) Maxim. Among these species, *A. holophylla* has a distinctive characteristic and can be easily identified by the acicular shape of the end of the needles and the hidden bract scale in the cone (Lee, 1985). However, many researchers have argued, based on their own opinions, which morphological characteristics between *A. koreana* and *A. nephrolepis* are and are not useful in identifying and delineating the species (Lee & Kim, 1982; Kim & Kim, 1983; Chang et al., 2000; Song et al., 2007; Song et al., 2008).

In general, plant species have a very weak sexual isolation, not only for firs, but also for other related tree species (Mergen et al., 1964; Perron & Bousquet, 1997; Isoda et al., 2000; Polezhaeva et al., 2010). Thus, in a natural environment, almost all plants can hybridise with each other. These hybrid individuals and introgressions with parent trees are regarded as a main factor of continuing variations of morphology in the plant kingdom (Jain, 1976; Parker et al., 1981; Parker et al., 1984; Hardig et al., 2000; Lanner, 2010). However, until now, few studies concerning firs in Korea have focused on the broad area of their distribution. Therefore, even if the possibility of hybridisation and introgression of firs in Korea makes no difference in morphological characteristics, pure populations of firs have to be investigated. In such cases, then, there are pure populations of *A. nephrolepis* in northeast China and far-east Russia and a pure population of *A. koreana* on Jeju Island, South Korea (Chŏng, 1943; Farjon, 1990; Jiang, 2010).

A. koreana is an endemic species that is mainly distributed on Jeju Island, which was formed by a volcanic event from the tertiary to quaternary periods in the Cenozoic era in the southernmost point of Korea (Kim, 2002; Lee et al., 2011). During the glacial time, Jeju Island was linked between the Korean peninsula and Japanese archipelagos. There is plentiful flora on Jeju Island; therefore, it is a historical treasure due to topographical and climatic events with the expansion and contraction of the plant range (Kong, 1998; Kong, 2004). Thus, an endemic taxon that is distributed on Jeju Island is considered a result of the glacial effects and needs to be examined with regard to its related species concerning their geographical range (Im, 1992).

In this study, when comparing the morphological characteristics of *A. koreana*, an *A. veitchii* complex, which ranged from Honshu to Sikoku in Japan, had been reported as an allied species. (Franco, 1950; Liu, 1971; Farjon & Rushforth, 1989). Thus, we investigated the different species together using the morphological characteristics, especially the resin duct, which is an evolutionary characteristic of fir species (Klaehn & Winieski, 1962)

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useful for the classification of other gymnosperms (Güvenç et al., 2011). Through this study, we wanted to understand the quantitative traits of resin ducts and their range of variation and confirm the range of variation of the resin duct to verify the characteristics of the resin ducts and, as such, to determine whether they could be used as good identification/determination keys.

2. Materials and methods

2.1. Plant materials

Taking into consideration the spatial-genetic structure of fir, *A. koreana* and *A. nephrolepis* samples were collected from areas about 50 m apart (Kim & Hyun, 2000). To identify the species based on previous studies, bract scale types were used as the standard by which to differentiate between these species. Sampling was carried out in 10 natural populations in China, Korea, and Russia (Figure 1), and from these taxa, 12–63 individuals per population were gathered (Table 1). For the *A. veitchii* complex samples, since it is neither indigenous to nor distributed throughout Korea, it was provided from the Uryu Research Forest of Hokkaido University.

2.2. Characters

In order to measure the quantitative traits of resin ducts, 10 needle leaves were selected from 2-year-old branchlets of each individual tree. Those needle leaves were first cut into 3 parts with a blade, and afterwards, only the middle parts of the needle leaves were prepared for use in a cross-

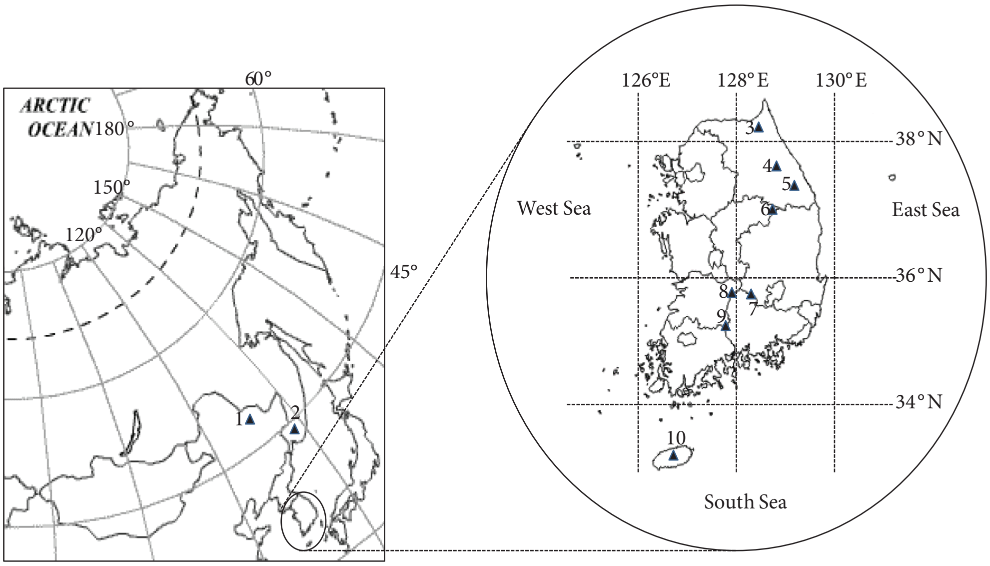

Figure 1. Overview of China, Russia, and South Korea with *Abies* sampling sites indicated with a triangle. Site numbers correspond to those of Table 1.

Table 1. *Abies koreana* and related firs' study sites and collection data in China, Russia, and South Korea.

		Location	Latitude	Longitude	Number of trees sampled
China	1	Liangshui Nature Reserve	47°10′N	128°53′E	50
Russia	2	Mts. Sikhote-Alin	44°01′N	134°12′E	12
South Korea	3	Mt. Seorak	38°07′N	128°22′E	25
	4	Mt. Jungwang	37°28′N	128°30′E	20
	5	Mt. Hambeak	37°12′N	128°54′E	27
	6	Mt. Sobeak	36°57′N	128°37′E	22
	7	Mt. Gaya	35°49′N	128°07′E	12
	8	Mt. Deogyu	35°47′N	127°39′E	22
	9	Mt. Jiri	35°13′–15′N	127°37′–50′E	36
	10	Mt. Halla	33°20′N	126°22′–37′E	63

section (Warren & Johnson, 1988). Each middle part was cut to a thickness of 60 μm using a microtome (Leica CM1850 Cryostat Cryocut Microtome, Leica Instruments, Germany). Each fragment of the needle leaves was fixed onto a slide glass with a modified fixative (2 × PVLG), and these slides were observed with a microscope (63×; Leitz, Weszlar, Germany). In addition, using a digital camera (Axio Imager, A1, Carl Zeiss, Germany), computer software Image J (Abràmoff et al., 2004), and Axio vision release 4.6.3 (Carl Zeiss) all quantitative traits were measured (Eo, 2012) (Table 2).

2.3. Data analysis

The average value per characteristic in an individual was used as the representative value of that individual. Each representative value per characteristic was used to compare the types of populations and species. To consider the morphological flexibility, we used ratio analysis. The values for the ratios were converted from the primary representative values, and then the ratios were analysed for the length and area of the resin duct (Robakowski et al., 2004). Here, VR was the resin duct portion from the lower to the upper epidermis of the needle leaves (② ÷ ④), HR was also a resin duct portion from the epidermis to the vascular bundle (① ÷ ③), and RLR was one portion of the resin duct area of a half cross-section area (Figure 2). Since the vascular bundle's position is almost consistent, this was used as a standard for measuring the traits of the resin duct (Roller, 1966). Thereafter, in order to determine the range in variation of the resin duct, univariate analysis was used to compare the morphological similarities and differences using a box plot of the populations and species. In addition, bivariate analysis was used to show some of the different associations of the morphological characteristics with each taxon using a scatter plot (Strandby et al., 2009).

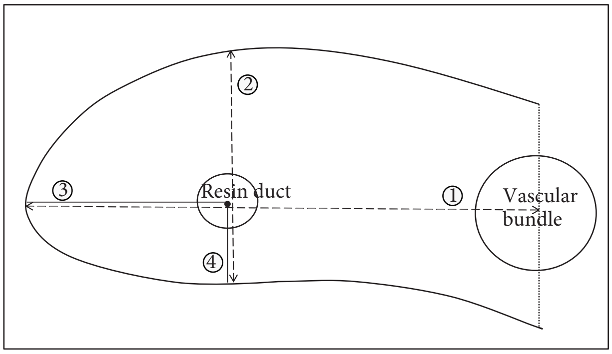

Figure 2. Anatomical structure of fir needle in a cross-section, showing numbers used in measuring a relative position of the resin duct.

3. Results and discussion

3.1. Morphological variation

Measured traits showed a general relationship with latitude (Figure 3). For example, the higher latitude populations had high values for VR and HR compared to the lower latitude populations. In comparing the sorts of taxa, *Abies nephrolepis* had high values for VR and HR compared to *A. koreana*. That is to say, the distribution patterns of *A. nephrolepis* and *A. koreana* had a strong connection with the range in variation. Thus, overall, from a higher latitude to a lower latitude, the resin duct position approached more and more the marginal part of the needle leaf. However, contrary to other studies, it was hard to find a perfect population, which would have a complete marginal resin duct type in the *A. koreana* populations. Of course, some individuals (Mt. Halla: 2, 10, 28, 31, 37) had complete contact with the marginal portion of the needle leaf; however, not even one Korean fir tree showed a consistent resin duct type that was marginal. Thus, this result means that the resin duct position has a morphological variation in the natural populations, and it also means that the range in variation can exceed the traditional resin duct type as

Table 2. Characters used in the morphological analysis. All characters are quantitative and were measured in micrometres.

Code	Character
NW	Needle width
NT	Needle thickness
DR	Diameter of resin duct
VR	Vertical position of resin duct from the lower needle surface (ratio)
HR	Horizontal position of resin duct from the needle margin (ratio)
RLR	Resin duct area / leaf cross-section area (ratio)

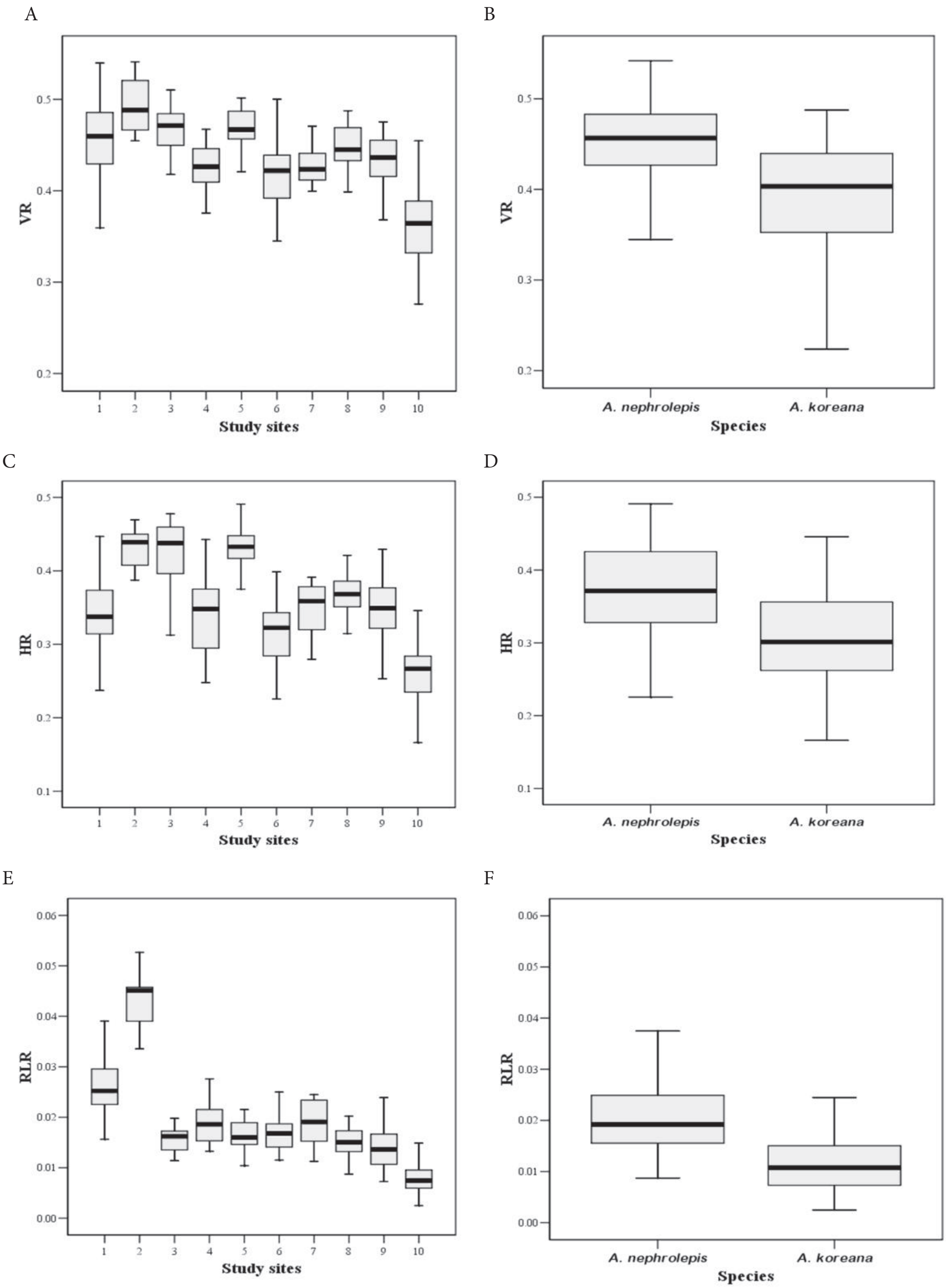

Figure 3. Characteristic variations between *Abies koreana* and *A. nephrolepis*. Site numbers of the x-axis correspond to those of Table 1 and abbreviations of the y-axis correspond to those of Table 2. Each y-axis value represents a ratio of resin duct characters.

marginal. For this reason, it is not appropriate for the resin duct position of *A. koreana* to be designated as a marginal type. Thus, a more suitable description is needed for the resin duct position of *Abies*. Hence, the previous resin duct type of *A. nephrolepis* is suitable as a median type; however, the identification of *A. koreana* has to change to include both the marginal type and the median type.

In this study, first, an investigation into the resin duct area (RLR), which was not dealt with in previous studies, was carried out. In Figure 3, similar to other resin duct traits, RLR also showed a clinal variation of sorts in the sampled populations and species. However, the populations in Lingshui Nature Reserve (China) and on Mt. Sikhote-Alin (Russia) had some distinguishable values for RLR when compared to populations from the Korean peninsula. Particularly, Mt. Sikhote-Alin had no reiteration with any populations in Korea. This is a unique value when comparing the VR and HR values of other populations from the Korean peninsula. Among the quantitative traits of the resin duct, the RLR clearly shows the association of morphological variation with latitude better than other traits, except in the Chinese population. Moreover, after careful consideration, all the compared traits of the resin duct are reflected by the genetic properties of the fir. From that viewpoint, although Mt. Sikhote-Alin is at a lower latitude than the Lingshui Nature Reserve, *A. nephrolepis* was better preserved on Mt. Sikhote-Alin than in the Lingshui Nature Reserve.

A scatter plot was used to determine the relationship of the combined traits to find out whether they could act as an identification key (Figure 4). From the results, it is clear that the scatter plot of combined traits did not help to identify fir species. All traits had ranges of reiteration for each combination. Mainly, population numbers 6, 7, 8, and 9 affected the reiteration patterns. This result is in line with other studies on the sympatric distribution of *A. nephrolepis* and *A. koreana* and on the possibility of gene flow across a given geographic region (geocline); therefore, to investigate gene flow, a molecular study on these taxa is necessary.

Finally, it is very difficult to distinguish these taxa by just the type of resin duct since they have clinal variation in the shape of the resin duct. After all, the bract scale is considered a key characteristic of these taxa for identification in the field (Chang et al., 1997; Song et al., 2007), and taken all together, it is important to consider the geographical distribution pattern of these taxa, as well.

3.2. Relationship of allied species

In this study, *A. veitchii*, a related species of *A. koreana*, had a median type resin duct, which was a similar resin duct position to that of the Mt. Sikhote-Alin population. However, the RLR value was very different. The results show that *A. veitchii* has a similar RLR value to that of

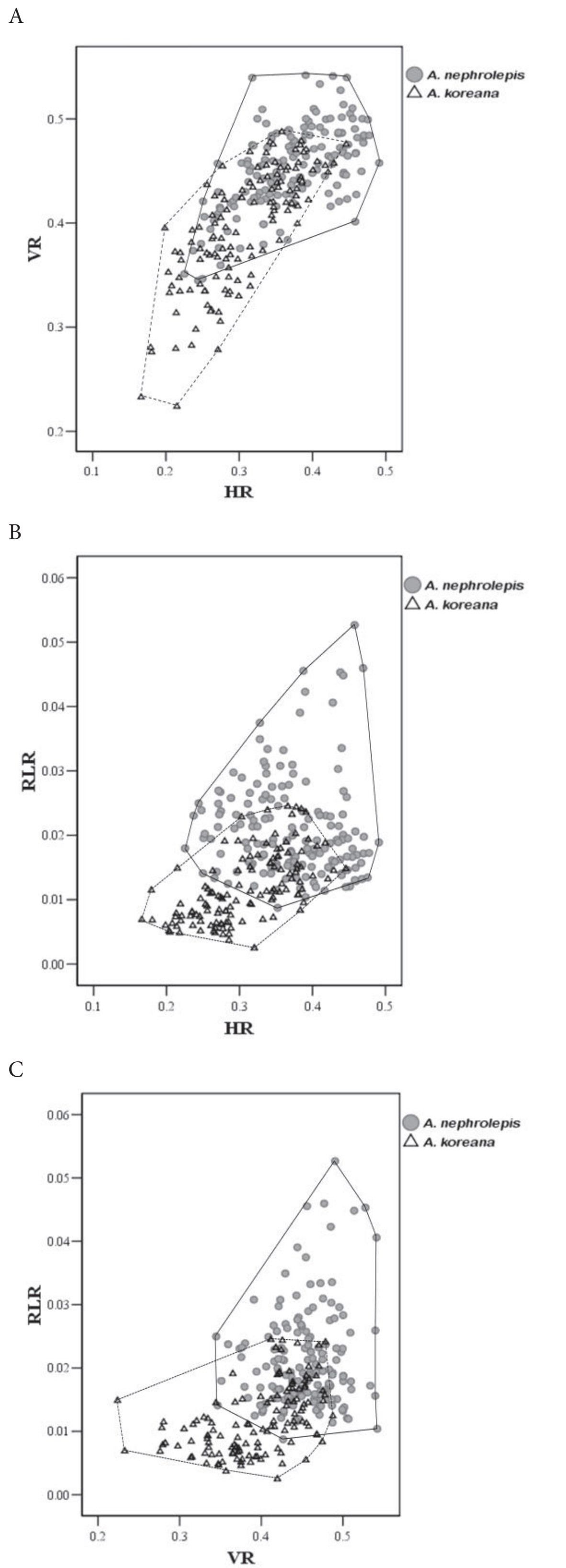

Figure 4. Scatter diagrams based on the 3 quantitative characters in the 289 sampled individuals. For abbreviations, see Table 2.

the Mt. Halla population. This value, however, does not represent the entire population of *A. veitchii* on the Japanese archipelagos since only 2 individuals were examined, and so there is still some uncertainty. However, it is possible to compare and postulate about the type of resin duct based on previous studies. In fact, the observations on the resin duct in this study and in Wilson's observation do not agree with other observations. In addition, while there are many descriptions of the resin duct of *A. veitchii,* there are also many confused results showing that the type varies from the median to the marginal type (Table 3).

As a matter of fact, *A. veitchii* has a broad distribution range, from Honshu (37°46′N) to Shikoku (33°44′N) (Yatoh, 1972). This distribution range is larger than that of *A. koreana*. Such a distribution pattern is often provided as support for the variation in morphological characteristics following changes in latitude (Tsugio & Iwamura, 1951; Park et al., 2010). Actually, from a literature survey, about 50% of the resin ducts of *A. veitchii* appeared to be the median type, while the other 50% were the marginal type. This will have to be further investigated to determine which resin duct shape is consistently expressed in this taxon.

Nevertheless, if we put together the analysis results for the types of resin ducts of *A. koreana* and *A. nephrolepis* in this study and from the results of the literature survey for *A. veitchii*, we can draw a tentative conclusion in line with the opinion of Warren and Johnson (1988) for the resin duct type of *A. veitchii* and also are able to apply this description to the resin duct type of *A. koreana*

In 1916, Koidzumi reported *A. veitchii* var. *reflexa* was distributed on Mt. Tsurugi and Mt. Ishizuchi in Shikoku. However, in 1925, he reported that *A. koreana* was on Mt. Tsurugi, too. However, after that, he had no further taxonomical comments on the taxon and their relationship to Mt. Tsurugi. In 1928, Nakai reported that *A. sikokiana*

Table 3. Given list of the results from previous studies of resin duct types.

Species	*A. nephrolepis*	*A. koreana*	*A. veitchii*	Author
	Median	Marginal	Marginal	Rehder, 1927
	Median	Median	Median	Franco, 1950
	Median	Median (sub)	Median	Liu, 1971
	Median	Median	Median to submarginal	Warren & Johnson, 1988
	Median	Median	Median	Farjon, 1990
	Median	Median	Submarginal	Eckenwalder, 2009
	Median	Submarginal	-	Klaehn & Winieski, 1962
	Median	Median (sub)	-	Lee, 1985
	Median	-	-	Cheng & Fu, 1978
	Median	-	-	IB-CAS, 1987
Resin duct type	-	Marginal	-	Wilson, 1920
	-	-	Marginal	Wilson, 1916
	-	-	Median	Murakoshi, 1942
			Median	Iwata, 1954
	-	-	Median	Kurata, 1964
			Median	Yatoh, 1972
	-	-	Marginal	Ohwi, 1984
	-	-	Marginal (sub)	Kitamura, 1984
	-	-	Median	Makino, 1988
	-	-	Median	Ohba, 1995
	Median	Median to marginal	-	Present study

was distributed on Mt. Tsurugi and Mt. Ishizuchi, in the same region reported by Koidzumi. Both of them explained that the bract formed the key characteristic of the taxon, but it is not clear from their works how to recognise the taxon characteristically. Moreover, it is necessary to consider that they gave a different name to the taxon based on their observations (Yamanaka, 1991). Thus, until now, the taxa distributed in this region are treated mainly as a variety of *A. veitchii*: either *A. veitchii* var. *sikokiana* or as a relative of *A. veitchii*, including *A. koreana*, based on Koidzumi in both taxa.

These 2 treatments are supported in the literature. Liu (1971), Farjon (1990), and Iwatsuki et al. (1995), and others supported the establishment of ranking the varieties, but Wilson (1916), Yatoh (1972), and Eckenwalder (2009), and others supported not making any ranks, instead wanting an inclusion of *A. veitchii* in and of itself. Taken together, their description focuses on the bract scale of the cone and the resin duct of the needle leaf. *A. veitchii* has a short exerted bract and is slightly recurved in Honshu; however, in Shikoku, it has a hidden or short exerted bract (Liu, 1971; Yatoh, 1972; Kitamura & Murata, 1984; Farjon, 1990). Moreover, it is considered that the resin duct type has gradually transformed from the median type in Honshu to the submarginal type in Shikoku (Liu, 1971). Therefore, considering the morphological variation following latitude, the Shikoku region can be regarded as the southernmost area of *A. veitchii*, and it can be inferred to have an extreme value for morphological variation. However, it helps to survey all of the morphological characteristics of the resin duct and bract scale in all distributions to confirm the rank of the taxon.

4. Conclusion

It is considered as a matter of course that a plant's distribution and a political frontier do not coincide generally. Many researchers have long recognised that an analysis of morphological characters of a plant should be performed in a well-prepared situation, which means collecting a target plant throughout the most distribution area. To date, many resin duct data of *A. koreana* and *A. nephrolepis* have been published, but the results were not totally acceptable because of the deficiencies in sampling sites and in comparing each character in detail. However, the current research can offer valuable information on how to explain the resin duct characters of firs in Korea on a morphological basis; the resin duct characters have a clinal variation and show an interspecific continuity in both species. Thus, for identification of these species, a bract scale is a better choice in the field. In addition, we were also able to try to guess an overlapping area of these species by the resin duct data. This study draws attention to the fir trees of Korea and Japan, indicating that further studies investigating the fir trees by morphological and molecular approaches, such as, for example, a mitochondrial DNA analysis to detect an introgression of mother trees, are necessary. Further scientific studies should be conducted to analyse the distribution pattern and gene flow of fir trees from a phytogeographical position.

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