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EFFECT OF LATITUDE AND LONGITUDE ON QUANTITATIVE CHANGES OF SOME ANATOMICAL AND MORPHOLOGICAL FEATURES OF ALNUS SUBCORDATA C. A. MEY. LEAVES IN HYRCANIAN FORESTS

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The effect of ecological factors and their interactions on quantitative changes of 20 anatomical and two morphological traits of leaves in six different populations of *Alnus subcordata* C. A. Mey. in the west and east of the Hyrcanian forests in three elevation classes include lowland, midland and highland were analyzed and compared. Leaf area, glandular trichomes, thickness of cuticle cells, and stomatal density decreased from west to east and were significantly different in three classes of altitude. There is a positive correlation between traits. Leaf thickness and thickness of adaxial epidermal cells, from west to east, were also significantly influenced by the interaction of ecological features. The decrease rainfall from west to east and altitude have affected the mentioned anatomical traits. Thickness and width of palisade cells, thickness of spongy cells, number of spongy layers and the thickness of the main veins from east to west were not significant, but in terms of elevations, their differences were significant. Also, there was a positive correlation between leaf area, stomatal density, glandular trichome maximal length, cuticle thickness and vein thickness. Number of adaxial collenchymas layers of vein, thickness of vein abaxial parenchyma cells, number of abaxial parenchyma layers of vein and thickness of vascular bundle of vein also increased significantly as altitude increased. Various types of stomata have also been observed in this species.

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Key words: Leaf anatomy; morphology; altitude; rainfall; Guilan; Golestan; Iran

اثر طول و عرض جغرافیایی بر تغییرات کمی برخی از ویژگی‌های آناتومیکی و مورفولوژیکی برگ گونه Alnus subcordata C. A. Mey. در جنگل‌های هیرکانی

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اثر عوامل اکولوژیکی و میان‌کنش آنها بر تغییرات کمی ۲۰ صفت آناتومیکی و دو صفت مورفولوژیکی در شش جمعیت مختلف توسکای ییلاقی (Alnus subcordata C. A. Mey.) در غرب و شرق جنگل‌های هیرکانی در سه طبقه ارتفاعی پایین‌بند، میان‌بند و بالابند بررسی و مورد مقایسه آماری قرار گرفت. صفات سطح برگ، کرک‌های غده‌ای، ضخامت سلول‌های کوتیکولی و تراکم روزنه‌ها از غرب به شرق کاهش و در سه طبقه ارتفاع از سطح دریا اختلاف معنی‌دار داشته و بین صفات نیز همبستگی مثبت وجود دارد. ضخامت برگ و ضخامت سلول‌های اپیدرم فوقانی از غرب به شرق روند افزایشی و میان‌کنش ویژگی‌های اکولوژیکی نیز معنی‌دار بوده است. ضخامت و پهنای سلول‌های نردبانی، ضخامت سلول‌های اسفنجی، تعداد لایه‌های اسفنجی و ضخامت رگبرگ اصلی از شرق به غرب معنی‌دار نبوده است، اما اختلاف آنها از نظر ارتفاع از سطح دریا معنی‌دار هستند. همچنین، همبستگی مثبت بین سطح برگ، تراکم روزنه، طول کرک، ضخامت کوتیکول با ضخامت رگبرگ نیز دیده می‌شود. تعداد لایه‌های کلانشیم رگبرگ اصلی در سطح تحتانی، ضخامت سلول‌های پارانشیمی و تعداد لایه‌های آن در سطح تحتانی و ضخامت دستجات آوندی در رگبرگ اصلی نیز با تغییر ارتفاع از سطح دریا افزایش معنی‌دار داشته است. تیپ‌های مختلف روزنه نیز در این گونه مشاهده شده است.

INTRODUCTION

Environmental factors influence the morphology of plants and determine the physiological function of plants. Leaves play a fundamental role in plant functions and their long-term adaptation to the local environment and climate. Although all leaves are similar in structural characteristics such as epidermis, stomata and mesophyll, but they differ among species in terms of surface size, thickness, shape and anatomical features (Hill & al. 2015; Tian & al. 2016). These morphological and anatomical variations or phenotypic flexibility occurred in response to environmental effects and is recognized as one of their most important adaptations to the environment (Thadani & al. 2009).

The structural changes of leaves in plants in different habitats have long been studied. The most common morphological changes in leaves are resizing the surface for photosynthesis. As a result of drought, the leaf area is reduced in order to increase moisture and increase photosynthetic efficiency (Franks & Beerling 2009; Thadani & al. 2009; Pyakurel & Wang 2014; Hill & al. 2015). The size of leaf area also varies with the amount of rainfall, temperature, light intensity and soil factors (McDonald & al. 2003; Meier & Leuschner 2008; Xu & al. 2009; Hill & al. 2015).

Anatomical variations of stomata in leaves and between leaves of different plants and populations have been studied and compared over the years (Salisbury, 1927; Shearman & Bearfd 1972; Lugg & Sinclair 1979; Solárová & Pospišilova 1983; Poole & al. 1996; Weyers & Lawson 1997). The mechanism of stomatal opening and closing can be variable even in different parts of the canopy of a plant (Eensalu & al. 2008). A variety of environmental factors such as the amount of water available in the soil (Pääkkönen & al. 1998; Limin & al. 2007), the light, the amount of ozone (O_3) and nutrients (Frey & al. 1996; Thadani & al. 2009) influence the size and density of the stomata. The stomata regulate the balance between water loss through transpiration and CO_2 fixation in photosynthesis (Al Afas & al. 2006), and even when soil moisture is not a limiting factor for plant growth, the stomata prevent leaf water deficiency, especially in tall trees (Camargo & Marenco 2011). Although humidity and temperature are two important factors in the formation of a climate, but altitude as well as sunlight intensity and geographical directions can influence it (Shayanmehr & al. 2014). Individuals of a population, especially trees, with different types of morphological and anatomical features at different elevations with temperature and precipitation fluctuations can protect themselves against the long-term effects of climate change (Valladares 2008).

Alder is the common name of a genus of flowering plants (*Alnus* Mill.) belonging to the birch family Betulaceae. The genus comprises of monoecious trees and shrubs, distributed throughout the north temperate zone with a few species extending into Central America, as well as the northern and southern Andes (Chen & Li 2004). *Alnus subcordata* C. A. Mey. is one of the relict species of third geological era in the Hyrcanian province, which as a pioneer species, it plays an important role in the vegetation structure of this unique area in the world (Hamzeh'ee & al. 2008; Akhani & al. 2010). Hyrcanian forests are usually defined in three altitudinal belts include lowland, midland and highland. *A. subcordata* is distributed throughout these forests at altitudes below 40 m and above 2000 m above sea level. The amount of rainfall in these forests usually decreases from west to east and the annual temperature increases (Zohary 1973; Naqinezhad & al. 2008; Siadati & al. 2010). There is not much anatomical study of *A.subcordata* despite its different climatic and soil conditions. Zhi-Duan & Zhi-Yun (1991) have been studied the anatomical characteristics of leaves in the Betulaceae family. Shayanmehr & al. (2014) have been studied morphological diversity of Alder species in northern Iran. Changes of density and size of stomata in *A. subcordata* have been studied in the Shast Kalate area of Golestan provience (Shirazi 2015). Due to the highly diverse ecological conditions in Hyrcanian forests and the results obtained from the impact of ecological factors, especially altitude on plant functional traits (Jafari & al. 2014; Jafari & al. 2015) this paper seeks to determine whether changing environmental conditions (altitude, temperature and rainfall) are effective in quantitative leaf anatomical characteristics in different *Alnus subcordata* populations? Will all the studied anatomical features respond differently to environmental factors?

MATERIAL AND METHODS

Study areas

This study was carried out in six areas (stations) of Hyrcanian forests, from west to east, in two northern provinces of the country, in Guilan and Golestan provinces, in three altitudes of 135 to 500 m (lowland), 930 to 1006 m (midland) and 1,650 meters (highland) above sea level. The geographical location and ecological features of the study areas are presented in fig. 1 and table 1 respectively. At each station along the southern slope, five trees of *A. subcordata*, approximately similar in age, were selected 100 m apart (Danquash & al. 2011). Then, five leaves were randomly collected, at full maturity without infection, for anatomical studies and 15 leaves for morphological measurements from the outermost half of the canopy on the south side of the trees.

Fig. 1. Geographical location of the studied areas.

Table 1: Geographical and ecological characteristics of six populations of *Alnus subcordata* in the studied stations (specimens are preserved in Ecology-Anatomy laboratory of Research Institute of Forests and Rangelands).

Site Abbreviation	Locality	Alt (m)	Longitude	Latitude	Annual percipitation (mm)	Annual temperature (○C)
GiAsL	Gilan: Asalem to Khalkhal, Lowland, Abbas-Azimi, Naqinezhad and Mohebi.	135	48°53'319"	37°42'231"	1958	15.600
GiAsM	Gilan: Asalem to Khalkhal, Midland, Abbas-Azimi, Naqinezhad and Mohebi.	1006	48°48'719"	37°40'053"	1286.5	8.500
GiAsH	Gilan: Asalem to Khalkhal, Highland, Abbas-Azimi, Naqinezhad and Mohebi.	1650	48°48'541"	37°37'460"	888.09	8.630
GTsL	Golestan: Gorgan, Tuskestan way, Lowland, Abbas-Azimi, Naqinezhad and Mohebi.	500	54°34'033"	36°44'418"	651.3	13.867
GTsM	Golestan: Gorgan, Tuskestan way, Midland, Abbas-Azimi, Naqinezhad and Mohebi.	930	54°35'020"	36°43'035"	266	13.700
GTsH	Golestan: Gorgan, Tuskestan way, Highland, Abbas-Azimi, Naqinezhad and Mohebi.	1650	54°34'930"	36°41'919"	198	12.100

Anatomy and morphology

Leaf area was measured using a leaf area meter (Light. AOX. 230V,. GATEHOUSE , U.K) in square centimeters. Leaf thickness was measured by a vernier caliper in millimeters from the lower half of the leaf and the distance between veins. For anatomical studies, the fresh leaves were fixed in 70% ethanol. Cross sections were made from the middle part of the leaf and from the middle vein area hand using commercial blades. The cuttings were cleared with sodium hypochlorite and then washed in several of distilled water. The sections were double stained with Carmine and Methylene blue colors and mounted in gelatin. To study of the density and length of the stomata and glandular trichomes a piece of the leaf epidermis was separated and cleared with sodium hypochlorite. Five replicates were randomly assigned to measure the dimensions of the stomata and trichomes in each slide. After preparing the slides, anatomical traits were measured using an Olympus BH2 RFCA light microscope. Pictures were taken by light microscope Olympus CH30 and Nikon digital camera model COLPIX P90. A total of two morphological and 20 anatomical traits have been analyzed statistically using common terms in anatomy (Metcalfe & Chalk 1950). Morphological traits include leaf area and leaf thickness and anatomical traits including abaxial stomatal density, abaxial stomatal length, abaxial trichome density, abaxial trichome maximal length, thickness of adaxial cuticle cells, thickness of abaxial cuticle cells, thickness of adaxial epidermal cells, thickness of abaxial epidermal cells, width of palisade cells, thickness of palisade cells, thickness of spongy cells, number of spongy layers, thickness of main vein, thickness of vein adaxial collenchymas, number of adaxial collenchymas layers of vein, thickness of vein abaxial collenchymas, number of abaxial collenchymas layers of vein, thickness of vein abaxial parenchyma cells, number of abaxial parenchyma layers of vein, thickness of vascular bundle of vein. The studied traits and their abbreviations are presented in table 2.

Table 2. Anatomical and morphological traits and abbreviations.

Traits	Abbreviations
Leaf area (cm^2)	LeAr
Leaf thickness (mm)	LeT
Abaxial stomatal density (no. mm^2)	AbStD
Abaxial stomatal length (µm)	AbStL
Abaxial glandular trichome density (no. mm^2)	AbGTrD
Abaxial glandular trichome maximal length (µm)	AbGTrML
Thickness of adaxial cuticle cells (µm)	TAdCu
Thickness of abaxial cuticle cells (µm)	TAbCu
Thickness of adaxial epidermal cells (µm)	TAdE
Thickness of abaxial epidermal cells (µm)	TAbE
Width of palisade cells (µm)	WP
Thickness of palisade cells (µm)	TP
Thickness of spongy cells (µm)	TS
Number of spongy layers	NS
Thickness of main vein (µm)	TV
Thickness of vein adaxial collenchymas (µm)	TVAdCo
Number of adaxial collenchymas layers of vein	NAdCoV
Thickness of vein abaxial collenchymas (µm)	TVAbCo
Number of abaxial collenchymas layers of vein	NAdCoV
Thickness of vein abaxial parenchyma cells (µm)	TVAbPar
Number of abaxial parenchyma layers of vein	NAbParV
Thickness of vascular bundle of vein (µm)	TVasV

Statistical analysis

After performing data normality test and homogeneity of variance test, mean and standard error were obtained for all traits at each site. Two-way analysis of variance was used to investigate the effects of province and altitude and their interactions on the studied variables (table 3). Correlation between variables was performed using Pearson's correlation analysis (table 4). All statistical analyses were conducted using the SPSS ver. 22 software package.

RESULTS AND DISCUSSION

As shown in table 3, Leaf thickness had a significant difference between provinces (longitude) and altitude (figs. 2A- C & 3D- E). Also the interaction of ecological factors on this trait was effective and significant. Leaf thickness is increasing from west (Guilan) to east (Golestan) as well as lowland to highland. With increasing elevation, temperature and precipitation has fallen in both provinces (table 1), but in Golestan province compared to Guilan province, the temperature increases and the amount of precipitation is significantly reduced. According to Pearson correlation analysis (P-valu <001), leaf thickness was also positively correlated with temperature (table 4). In other studies, leaf thickness has also increased with increasing temperature (Tian & al. 2016). Increasing leaf thickness is thought to reduces leaf absorptance, thereby reducing heat load and lowering leaf internal temperature and transpiration rate. In glabrous leaves, the thick leaves increase the absorption of near-infrared radiation by their thick epidermal cells before transmitting the radiation to the internal mesophyll of the leaf and thereby protect the leaves from injury. In other words, the epidermal cells also play a role in the internal protection of the leaf. Altogether, this type of environmental adaptation creates the optimum temperature for photosynthesis in the leaves (Liu & al. 2015). The thickness of adaxial epidermal cells in Golestan province had a significant difference with the thickness of adaxial epidermal cells in Guilan province (figs. 2 & 3). The thickness of adaxial epidermal cells increased along with leaf thickness and they have a positive correlation (P-value <001) with each other (table 4).

Epidermis thickness plays an important role in the differences in leaf thickness (Araus & al. 1986).

Also, Pearson correlation analysis (P-valu <001) shows a negative correlation of this trait with the amount of rainfall (table 3). Interestingly, in this species, the adaxial epidermal cells are bilayered (figs. 2C & 3F), whereas other tree species of Hyrcanian forests such as *Quercus macranthera* Fisch. & C. A. Mey. ex Hohen., *Fagus orientalis* Lipsky, *Carpinus betulus* L., *Diospyros lotus* L. and *Parrotia persica* (DC.) C. A. Mey. have a single layer epidermis (Amjadi & al. 2013; Sharafi & al. 2013). Cuticule, epidermis and glandular trichomes are considered as a mechanism to minimize transpiration water loss (Liu & al. 2015). *Alnus subcordata* has a glandular trichome (fig. 2B (which, unlike the thickness of the leaf and epidermal cells, its density and long length have a diminishing trend from west to east. Pearson correlation coefficient also showed a negative relationship between trichome density with altitude (P-valu <001) and a positive correlation with precipitation (P-valu <001) (table 3). Abaxial trichome maximal length was positively correlated with altitude and negatively correlated with temperature (P <0.001). The density and maximal length of glandular trichome were negatively correlated with leaf thickness (table 4). Thickness of adaxial cuticle cells also show a decreasing trend from west to east as well as glandular trichome density (table 3). According to Pearson correlation analysis, the thickness of adaxial cuticle cells was positively correlated (P <0.001) with the amount of rainfall (table 4). Increasing the thickness of epidermal cells seems to be a protective adaptation mechanism of this species against diminishing cuticular cover in hotter and drier conditions. Although in some cases the cuticle and epidermis thickness were not significantly correlated with altitude (Velízquez-Rosas & al. 2002). The width of palisade cells, thickness of palisade cells, thickness of spongy cells and the number of spongy layers (figs. 2A- C & 3D- E) from east to west (longitude) were not significantly different, but altitudinally (latitude) there was a significant difference (table 3). Variations of the above traits at different altitudes along with changes in leaf thickness have provided an interesting relationship. The width of the palisade cells increased from the lowland to the midland, and then there was a downward to the highland (fig. 3E - F). The thickness of palisade cells and the thickness of the spongy cells, like the thickness of the leaves, increased from the lowland to the highland (figs. 2 & 3). There was also a positive correlation between leaf thickness, palisade cell thickness, the thickness of spongy cell, cuticle cell thickness and epidermal cell thickness (P-valu <001 and P-valu <0.05) (table 4). It seems that according to the significant interaction of ecological factors such as temperature and percipitation, in this altitudinal range (table 2), ecological factors cause these changes. Correlation between width and thickness of palisade cells and thickness of spongy cells with altitude was positive and with annual percipitation was negative (P-valu <001 and P-valu <005) (table 4). In the studied trees in southern Mexico (Velízquez-Rosas & al. 2002), the leaf thickness, thickness of palisade cells and thickness of spongy cells were also positively correlated with elevation. In this study, leaf area decreased with increasing altitude as well as longtitude (west to east). The results of table 3 showed that the quantities of this trait were significantly different in the three altitudes studied. Pearson correlation coefficient (table 4) also shows that there is a positive correlation (P-valu <001) between this trait with altitude and precipitation. Other studies have shown this downward trend and have emphasized that the leaf area is strongly influenced by ecological factors, particularly altitude (Körner & al. 1989; Velízquez-Rosas & al. 2002; Meier & Leuschner 2008; Xu & al. 2009; Paridari & al. 2012). Photosynthesis, transpiration, respiration and light interception are directly related to leaf area and stomata density. Four types of stomatal apparatus on mature leaves including anomocytic, cyclocytic, non-typical actinocytic and brachyparacytic are recognized in genera of the Betulaceae (Zhi-Duan & Zhi-Yun 1991). Stomata type in *Carpinus betulus* was paracytic, anomocytic, and anisocytic and *C. oreintalis* were laterocytic and *C. schuschaensis* was anisocytic and laterocytic (Paridari & al. 2012). Based on the terms used by Nisa & al. (2019), the following stomata types including anomocytic, tetracytic, laterocytic (three subtypes include: L1, L2 and L3), brachyparacytic (two subtypes include: amphibrachy-paracytic and incomplete amphibrachyparacytic) and actinocytic were observed in *Alnus subcordata* (fig. 4).

The number and location of stomata in the leaf are important for crop production and plant water balance (Nurten & Aygün 2014).The results show that the density of stomata decreases from west to east. The density of the stomata along the elevation gradiant also decreased from lower altitudes to middle altitudes, and then there was an increasing trend towards higher elevations (table 3). According to table 1, the temperature decreased with increasing altitude in both study areas. In the high altitudes of Golestan province, precipitation is lower than the high altitudes in Guilan province, and the temperature is higher than

Guilan province (table 1). Pearson's correlation coefficient shows different relationships between stomata and trichome traits (table 4). There was a negative correlation between density and stomata length. There is also a negative correlation between stomata density and trichome density and a positive correlation between stomata density and trichome length. Stomata length is also positively correlated (P-valu <001) with trichome length. It seems that water deficiency is leading to an increase in stomata density (Ichie & al. 2015). Other Studies have also shown that stomatal variation in *Betula papyrifera* Marshal (Pyakurel & Wang 2014) and *Fagus orientalis* Lipsky (Bayramzadeh 2011) is due to habitat and ecological differences and stomata density is negatively correlated with its length (Zhang & al. 2012; Tian & al. 2016). In *A. subcordata*, like most forest trees, the stomata are located only on the abaxial leaf surface (hypostomy), but many herbaceous plants, especially weeds, have stomata on both surfaces (amphistomy). Coordinated evolution of adaxial stomatal density and light tolerance indicates that amphistomy is an important adaptation to optimally balance light acquisition with gas exchange (Muir 2018). There was a significant difference between the thickness of the main vein at different altitudes (table 2). The decrease in temperature occurs with increasing altitude and hence there is a negative correlation (P-valu <0.001) between the thickness of the main vein and the temperature. There was also a positive correlation between leaf area, stomata density, glandular trichome length, cuticle thickness and main vein thickness (table 4). Along with the thickness of the main vein, there was a significant increase in number of abaxial collenchymas, the thickness of vein abaxial parenchyma cells, number of abaxial parenchyma layers and the thickness of the vascular bundles in the main vein (table 3). Based on Pearson correlation analysis there is a positive correlation (P-valu <001 and P-valu <005) between the anatomical traits within the main vein including the thickness of vein adaxial and abaxial collenchymas and the number of layers, as well as the thickness of the main vein (fig. 2a & c). There was a positive correlation (P-valu <005) between these traits with stomata density, glandular trichome length and cuticle thickness. There is also a negative correlation (P-valu <005) between the thickness of the epidermis in the upper and lower surfaces and width and parenchymal thickness. There was also a positive correlation (P-value <005) between the thickness of the vascular bundles and the above trait (table 4). Thick cuticles, thick palisade cells and collenchymas and higher stomata density are usually features of plants that live in drier habitats (Thadani & al. 2009).

Conclusions

The results of this study showed that the quantitative anatomical traits of the *Alnus subcordata* are affected by rainfall and altitude. The impact of declining rainfall from west to east and increasing altitude on leaf area and its thickness are the anatomical-morphological features of most trees of Hyrcanian forests. The number of stomata has increased with altitude and has also decreased with increasing rainfall. It is also thought that there is a positive correlation between palisade and spongy tissues, and epidermis with leaf thickness. Also there is a negative correlation of these traits with leaf area, and the thickness of the main veins, the thickness of the vascular bundles as well as the number of adaxial collenchyma layers with increasing altitude. These quantitative anatomical changes indicate the specific functional performance of this plant in different ecological conditions. In most plants, in order to deal with dehydration, the cuticle thickness and trichome density increase in hot and dry conditions. While in *A. subcordata*, the density of trichome and thickness of cuticle has decreased in warmer and drier places (Golestan province and higher altitudes). Unlike other plants, *A. subcordata* seems to use the mechanism of double layering of the upper epidermis along with other traits to combat dehydration. Tall trees, even in conditions where soil moisture is not a limiting factor, face a reduction in hydraulic conductivity. It seems that the existence of different ecological conditions in Hyrcanian forests has led to morphological-anatomical mechanisms in *A. subcordata*, which indicate different genetic strategies to deal with water loss.

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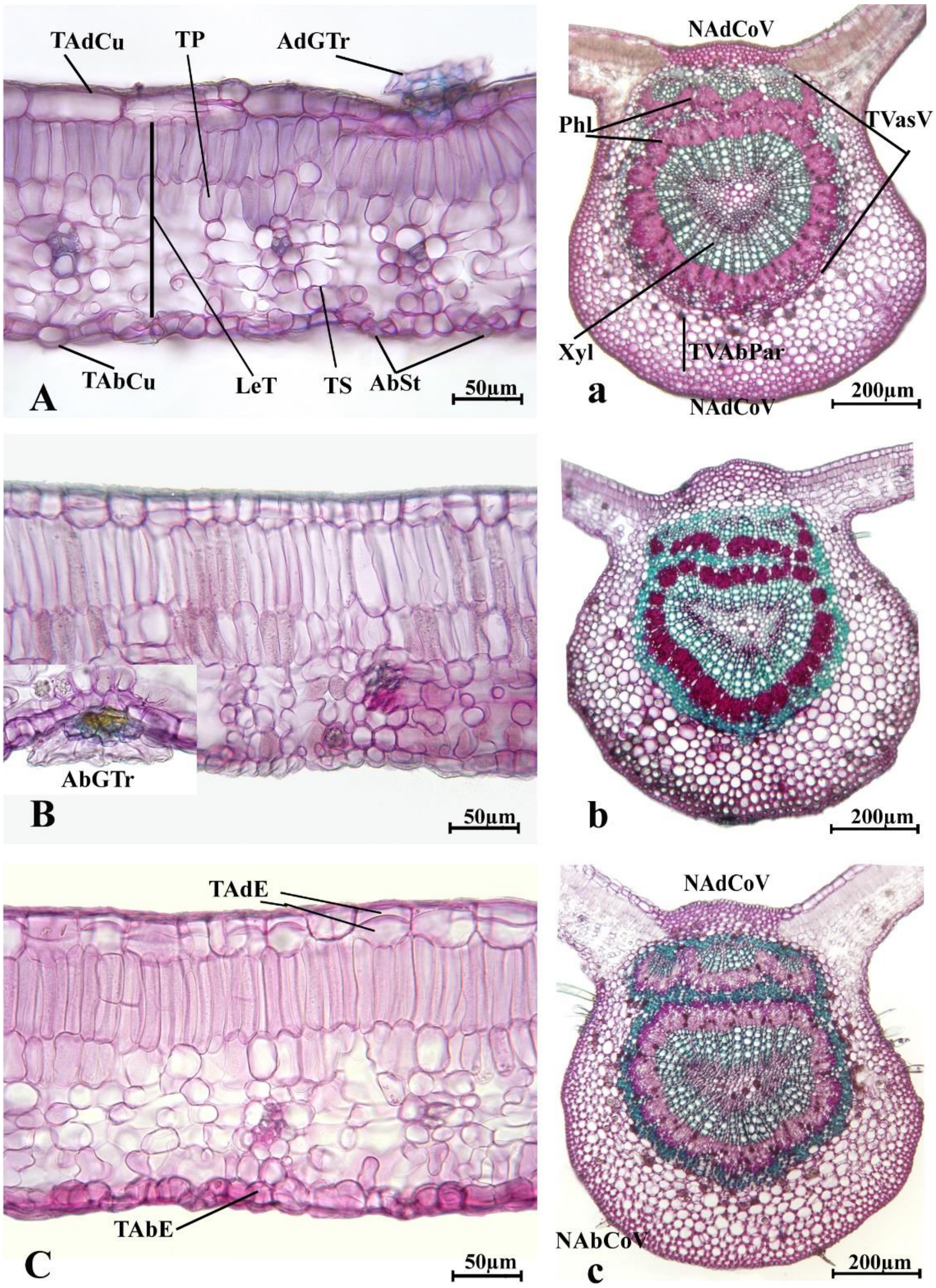

Fig. 2. Transverse section of leaves of *Alnus subcordata* populations. Guilan: lowland (A-a); midland (B, b); highland (C, c). Lamina: (A, B, C); midrib: (a, b, c). LeT= Leaf thickness; TAdCu= Thickness of adaxial cuticle cells; TAbCu= Thickness of abaxial cuticle cells; AbGTr= Abaxial glandular trichome; TAdE= Thickness of bilayered adaxial epidermal cells; TAbE= Thickness of abaxial epidermal cells; TP= Thickness of palisade cells; TS= Thickness of spongy cells; AbSt= Abaxial stomatal; NAdCoV= Number of adaxial collenchymas layers of vein; NAbCoV= Number of abaxial collenchymas layers of vein; TVAbPar= Thickness of vein abaxial parenchyma cells; TVasV= Thickness of vascular bundle of vein (Xyl=Xylem and Ph=Phloem).

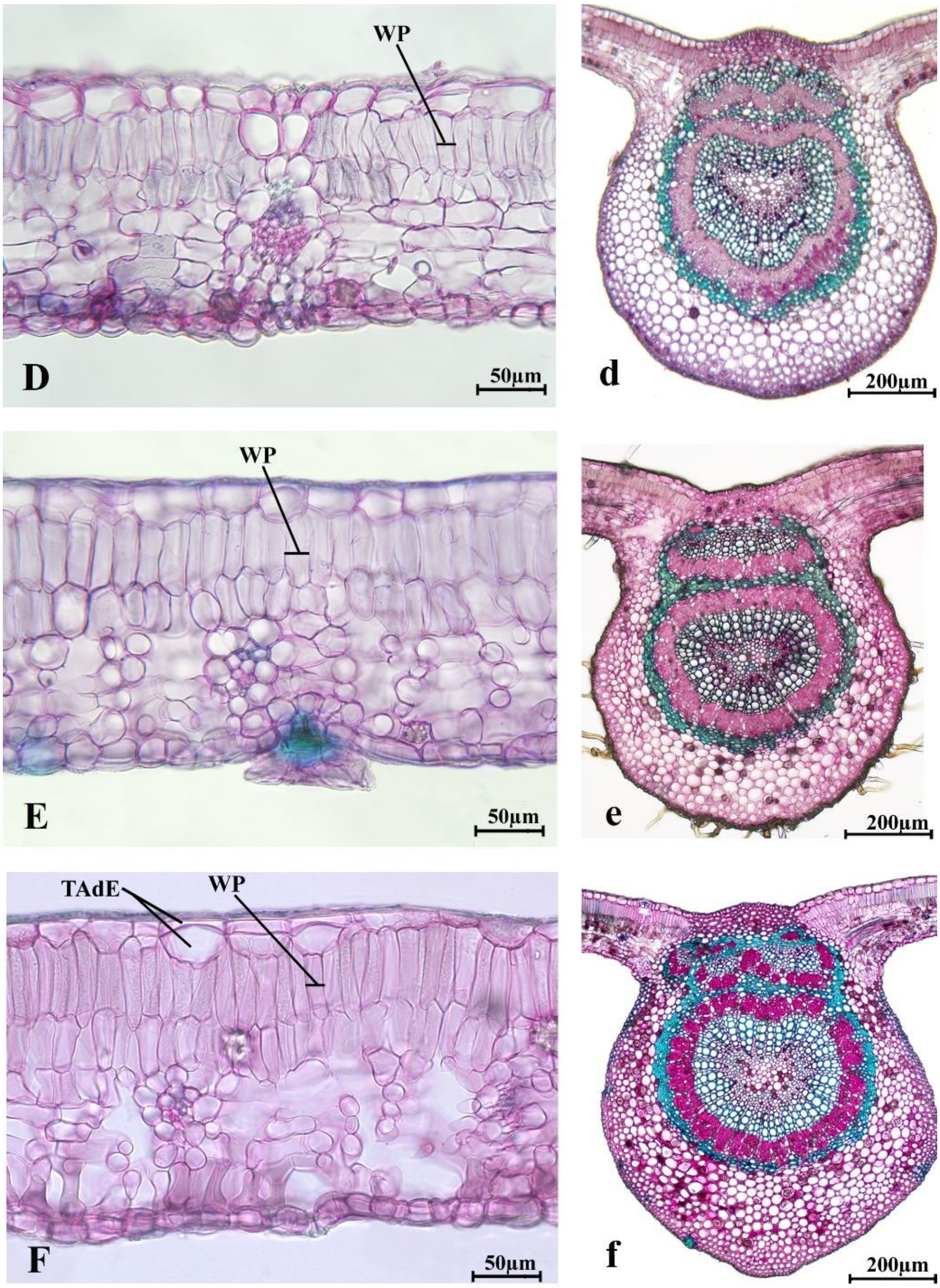

Fig. 3. Transverse section of leaves of *Alnus subcordata* populations. Golestan: lowland (D, d); midland (E, e); highland (F, f). Lamina: (D, E, F); midrib: (d, e, f). TAdE= Thickness of bilayered adaxial epidermal cells; WP= Width of Palisade cells.

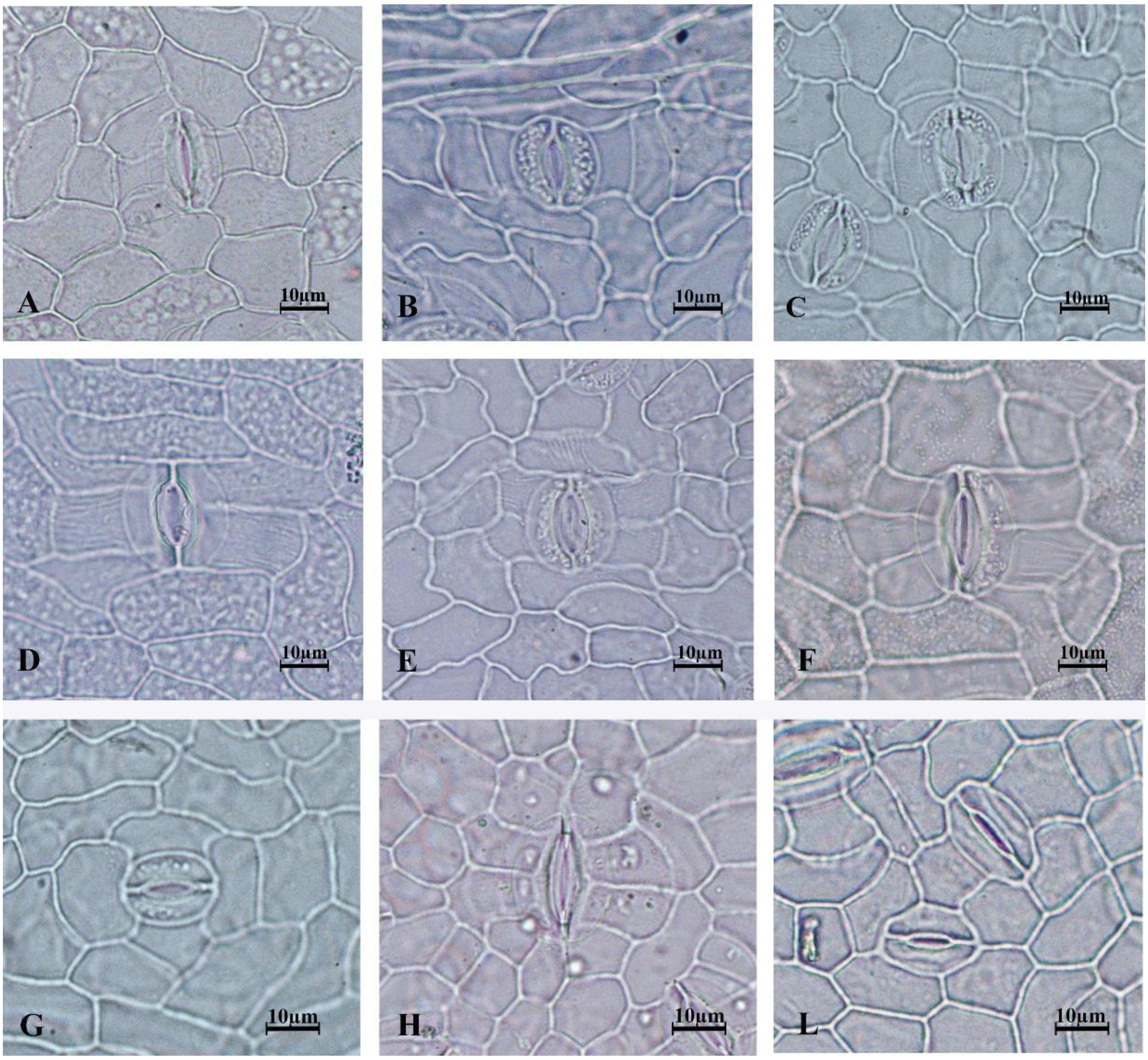

Fig. 4. Stomata types of the lower surfaces of leaves of studied populations of *Alnus subcordata* populations. A=Brachyparacytic; B=Amphibrachyparacytic; C=Incomplete amphibrachyparacytic; D=Laterocytic: L1 (2 +1); E=Laterocytic: L2 (2 +2); F=Laterocytic: L3 (2 +3); G=Tetracytic; H=Actinocytic; L=Anomocytic.

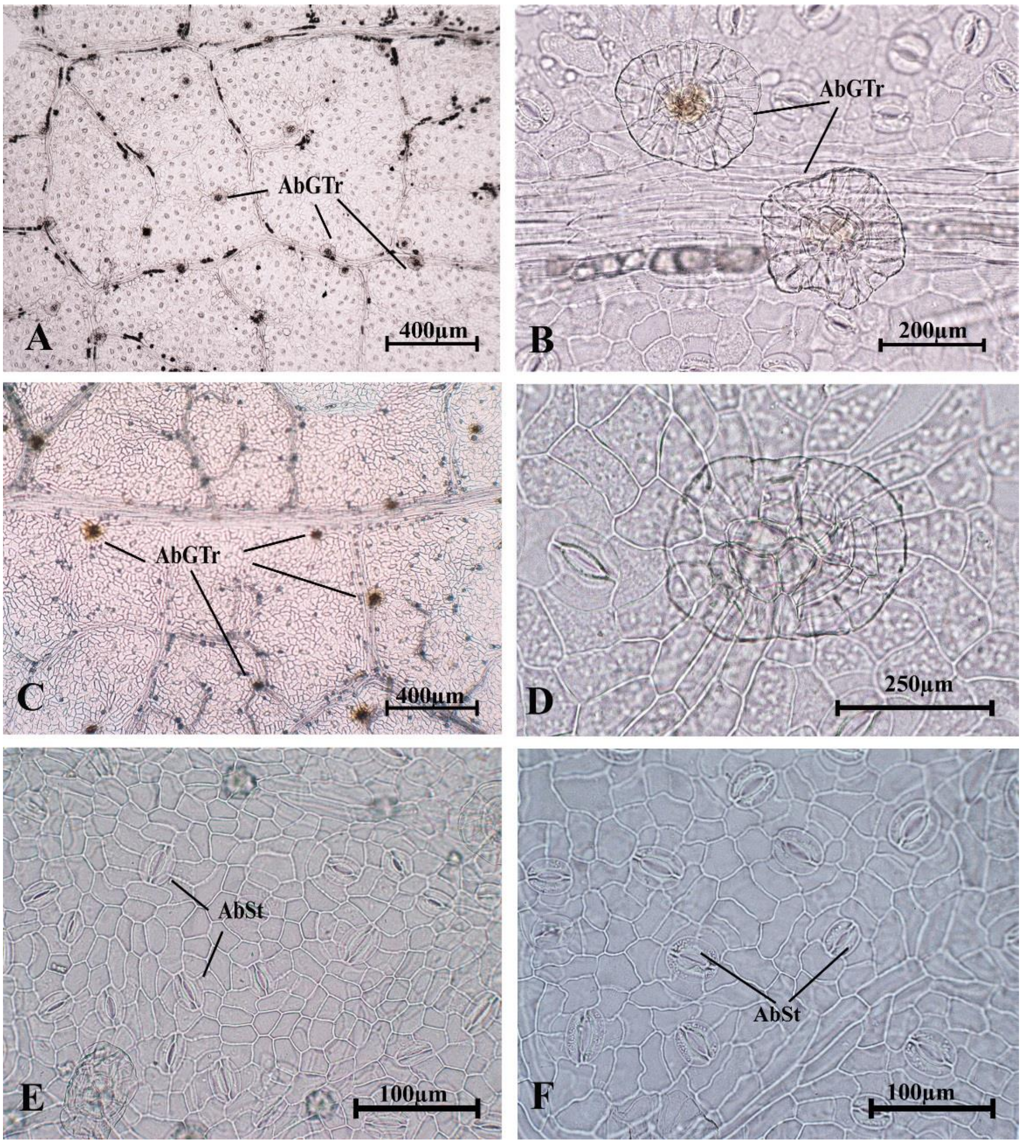

Fig. 5. Stomata and glandular trichome of the lower surfaces of leaves of *Alnus subcordata* C. A. Mey. Guilan (highland): A, B & E; Golestan (highland): C, D & F; AbGTr= Abaxial glandular trichome, AbSt= Abaxial stomatal.

Table 3. Mean, standard error and significant values of leaf anatomical and morphological traits in *Alnus subcordata* in Guilan and Golestan provinces, lowland, midland and highland and their interactions. P<0.05, <0.01 and <0.001 are identified as *, ** and *** respectively and ns is not statistically significant.

Trait	Mean± standard error					*P -value* (Province)	*P- value* (Elevational)	*P-value* (Province × Elevational)
	Gilan	Golestan	Lowland	Midland	Highland			
Leaf area (cm^2)	55.37±1.81	44.75±1.22	52.89±2.25	51.08±2.09	46.02±1.57	0.000***	0.032*	ns
Leaf thickness (mm)	0.13±0.002	0.18±0.003	0.16±0.01	0.17±0.01	0.18±0.00	0.000***	0.000***	0.000***
Abaxial stomatal density (no. mm^2)	188.84±4.73	174.85±4.91	175.54±6.13	171.08±5.91	199.13±5.13	0.033*	0.001**	ns
Abaxial stomatal length (µm)	29.01±0.29	32.26±0.30	30.17±0.48	30.47±0.42	31.31±0.37	0.000***	ns	ns
Abaxial glandular trichome density (no. mm^2)	76.87±0.61	72.39±0.71	76.32±0.81	74.95±0.87	72.54±0.87	0.000***	0.003**	0.000***
Abaxial glandular trichome maximal length (µm)	6.53±0.21	5.25±0.25	5.12±0.22	5.58±0.32	6.99±0.28	0.000***	0.000***	0.022*
Thickness of adaxial cuticle cells (µm)	4.70±0.12	4.35±0.09	4.48±0.14	4.76±0.14	4.33±0.10	0.013*	0.039*	0.006**
Thickness of abaxial cuticle cells (µm)	3.23±0.13	2.69±0.10	2.84±0.13	2.80±0.14	3.24±0.17	0.001**	0.039*	0.008**
Thickness of adaxial epidermal cells (µm)	20.19±0.52	23.04±0.41	21.44±0.64	21.96±0.69	21.47±0.48	0.000***	ns	ns
Thickness of abaxial epidermal cells (µm)	13.03±0.32	12.93±0.29	12.64±0.33	12.96±0.43	13.35±0.36	ns	ns	0.001**
Width of palisade cells (µm)	9.22±0.16	9.27±0.17	8.84±0.18	9.86±0.19	9.04±0.21	ns	0.000***	0.000***
Thickness of palisade cells (µm)	70.43±1.56	70.75±1.48	64.44±1.35	69.52±2.12	77.96±1.47	ns	0.000***	0.001**
Thickness of spongy cells (µm)	64.96±1.43	66.88±1.57	60.36±1.54	64.78±2.03	72.78±1.46	ns	0.000***	0.002**
Number of spongy layers	4.27±0.08	4.52±0.07	4.36±0.07	4.42±0.08	4.41±0.12	0.018*	ns	ns
Thickness of main vein (µm)	1100.3±18.9	1054.8±19.8	1006.9±18.8	1091.2±27.2	1135.2±21.4	ns	0.000***	ns
Thickness of vein adaxial collenchymas (µm)	81.35±2.02	77.87±2.46	72.36±2.99	83.48±2.42	83.02±2.58	ns	0.005**	ns
Number of adaxial collenchymas layers of vein	5.55±0.10	5.03±0.12	5.14±0.13	5.32±0.14	5.41±0.15	0.001**	ns	ns
Thickness of vein abaxial collenchymas (µm)	91.27±2.91	83.89±2.55	85.96±3.43	86.80±2.94	89.96±3.77	ns	ns	0.026*
Number of abaxial collenchymas layers of vein	4.05±0.11	3.81±0.11	3.82±0.11	3.60±0.13	4.39±0.15	ns	0.000***	0.002**
Thickness of vein abaxial parenchyma cells (µm)	139.89±4.40	134.19±4.94	133.84±6.44	126.72±4.68	150.78±5.48	ns	0.008**	ns
Number of abaxial parenchyma layers of vein	4.95±0.14	4.87±0.14	4.80±0.16	4.52±0.16	5.41±0.17	ns	0.001**	ns
Thickness of vascular bundle of vein (µm)	766.24±15.1	720.53±13.6	700.80±15.4	748.96±21.0	780.69±14.8	0.021*	0.005**	ns

Table 4. Pearson correlation analysis between anatomical traits of *Alnus subcordata* and ecological factors (full names of traits are given in table 2. P<0.05 and P<0.01 are identified as * and **).

	Alt	Pre Anu	Tem Anu	Le Ar	Le Th	Ab StD	Ab StL	AbG TrD	AbTr GML	Tad Cu	Tab Cu	Tad E	Tab E	WP
Alt	1													
PreAnu	-.598**	1												
TemAnu	-.708**	.091	1											
LeAr	-.235**	.415**	-.017	1										
LeTh	-.019	-.637**	.329**	-.179*	1									
AbStD	.212**	.044	-.176*	.007	-.146	1								
AbStL	.192*	-.521**	.170*	-.140	.463**	-.486**	1							
AbTrD	-.225**	.377**	-.158	.090	-.172*	-.218**	-.089	1						
AbTrML	.325**	.115	-.335**	-.048	-.294**	.526**	-.297**	-.075	1					
TAdCu	-.129	.227**	.034	.199*	-.169*	-.017	-.089	-.029	.087	1				
TAbCu	.096	.149	-.078	.163*	-.024	.164*	-.063	-.052	.211**	.234**	1			
TAdE	.020	-.308**	.128	-.091	.406**	-.099	.320**	-.123	-.180*	.083	.043	1		
TAbE	.087	-.060	-.004	-.256**	.134	-.273**	.145	.109	-.068	-.110	.095	.200*	1	
WP	.160	-.200*	-.283**	-.171*	.121	-.202*	.035	.020	-.182*	-.152	-.144	.044	.144	1
TP	.375**	-.203*	-.128	-.020	.202*	.167*	.273**	-.114	.188*	.032	.357**	.245**	.214**	-.146
TS	.362**	-.240**	-.101	-.067	.255**	.100	.298**	.061	.163*	-.028	.296**	.125	.304**	-.130
NSL	.039	-.158	.093	-.068	.093	.030	.109	-.030	-.135	-.152	-.072	-.004	.066	.131
TV	-.303**	-.073	-.315**	.318**	.044	.360**	.056	-.054	.216**	.029	.180*	.127	-.200*	-.142
TVAdCo	.211**	-.051	-.230**	.149	-.115	.251**	-.120	-.109	.159	.116	.055	-.059	-.101	.061
NAdCoV	.066	.185*	-.123	.232**	-.186*	.329**	-.167*	-.069	.231**	.001	.117	-.238**	-.188*	-.119
TVAbCo	.021	.104	-.003	.328**	.011	.239**	-.031	-.066	.072	.151	.238**	.039	-.083	-.193*
NAbCoV	.175*	.065	-.022	.198*	-.120	.255**	-.032	-.133	.310**	.181*	.281**	-.033	-.196*	-.327**
TVAbPar	.171*	-.028	-.116	.251**	-.009	.353**	-.043	-.121	.062	-.152	-.028	.059	-.119	-.144
NAbParV	.196*	-.051	-.074	.148	-.036	.322**	-.054	-.188*	.148	-.054	-.025	-.015	-.136	-.219**
TVasV	.246**	.005	-.293**	.307**	.009	.314**	.066	-.005	.266**	.070	.258**	.118	-.131	-.153

Table 4. Continued

	TP	TS	NSL	TV	TVAdCo	NAdCoV	TVAbCo	NAbCoV	TVAbPar	NAbParV	TVasV
TS	.637**	1									
NSL	.017	.337**	1								
TV	.338**	.283**	.029	1							
TVAdCo	-.032	.031	.129	.542**	1						
NAdCoV	-.014	.091	.182*	.503**	.609**	1					
TVAbCo	.235**	.167*	-.090	.582**	.489**	.408**	1				
NAbCoV	.301**	.225**	-.081	.439**	.262**	.306**	.621**	1			
TVAbPar	.105	.090	.068	.607**	.329**	.312**	.355**	.132	1		
NAbParV	.180*	.167*	.153	.439**	.151	.231**	.080	.150	.689**	1	
TVasV	.440**	.297**	-.002	.880**	.411**	.361**	.463**	.382**	.441**	.330**	1

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