Anatomical Features of Branchwood and Stemwood of *Betula costata* Trautv. from Natural Secondary Forests in China

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To enhance effective wood utilization, knowledge of the anatomical features that impact its service behavior is indispensable. The anatomical features of branchwood and stemwood of *Betula costata* Trautv. from natural secondary forests in central (Muzhaling mountain) and northeast (Maoershan mountain) China were studied to provide adequate information to enhance their efficient utilization, especially branchwood, whose use could widen the raw material base of the timber industry. Microtomed sections were employed to determine the tissue dimensions and proportions. Analyses of variance were used to test the anatomical feature differences between the two different sites, between the stemwood and branchwood, and between the heartwood and sapwood. The results showed that *B. costata* wood is diffuse-porous with more but narrower vessels located in the branch than in the stem. The branchwood also had a significantly higher fiber proportion than the stemwood. The sapwood exhibited significantly longer fibers than the heartwood. *B. costata* from Maoershan had significantly longer fibers, lower fiber proportions, larger fiber lumen diameter, and higher vessel density than that from Muzhaling. The results suggested that *B. costata* branchwood from Maoershan is suitable for papermaking and glued plates, while stemwood can be used for light construction purposes.

Keywords: Anatomical features; Betula costata Trautv; Branchwood; Stemwood

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INTRODUCTION

The global forest industry produces a large amount of branchwood, which represents an important secondary wood resource (Gurau *et al*. 2009). However, most branches are left as fuel or even waste when commercial harvesting operations concentrate their interest on large and straight stems. Branchwood utilization in wood industries is limited by its small size and unknown wood properties. Special focus has recently been placed on branchwood to meet the increasing industrial demands and environmental pressures because of the decline in stemwood resources (Leitch and Miller 2017; Dadzie *et al*. 2018). Branchwood can be engineered into a number of products, such as lumber (Okai and Boateng 2007), kraft pulp (Boyle and Ek 1972), and scrimber (Yu *et al*. 2015).

The prerequisite for efficient utilization of branchwood is to have a full understanding of its structure and properties, especially its anatomical features that are often responsible for the physical and mechanical properties (Adeniyi *et al*. 2013; Zhao *et al*. 2018). However, the arrangement, distribution, and size of wood elements are not the same for branches and stems. Many researchers believe that branches have smaller wood elements than stems (Zhao 2015; Longui *et al*. 2017), which might be one of the major limits of branchwood application. However, the different anatomical features exhibited by wood have made it useful in different fields of application. Long fibers are extremely beneficial for improving the fracture toughness of paper (Larsson *et al*. 2018), while short fibers result in good mechanical properties in wood plastic composites, as they are easier to disperse in high-density polyethylene matrices (Wan Mohamed *et al*. 2018). Wood types with thin-walled fibers and large pores have a low density and strength and are therefore preferred for light construction purposes, whereas wood specimens with thick-walled fibers have a high density and strength, which are useful in heavy construction work (Adeniyi *et al*. 2013). Wood types with large rays offer a fine texture when quarter-sawn, but can be split easily when nailed at the ends. Wood with multiseriate rays has better transverse penetration. Furthermore, the high proportion of ray tissue can decrease the radial shrinkage (Xue *et al*. 2018) and increase the radial strength of the wood (Burgert and Eckstein 2001). In some ways, the processing performance of branchwood might be even better than that of stemwood. For example, *Entandrophragma cylindricum* is more durable than its stemwood counterpart (Dadzie *et al*. 2016).

Betula costata Trautv., which originated in eastern Siberia, has become intensively distributed throughout China and the coastal areas of Russia, and is also found in North Korea, Mongolia, and Japan (Kochergina *et al*. 1987; Chen *et al*. 2000). *B. costata* stemwood is hard and uniform in structure, which is ideal for drying and processing. This suggests that it has potential to be used as a timber source (Yang *et al*. 2013). *B. costata* wood is also widely used for the manufacture of pulp, paper, and other fiber-based products. Regrettably, this species is rarely cultivated, and most *B. costata* stemwood is from natural virgin forests or secondary forests (Ni 1985). *B. costata* forest is one of the typical community types in the secondary succession of broad-leaved Korean pine forest in China, especially in Northeast China, where it comprises about 20% of the forest composition (Chen *et al*. 2000). With the increasing demand for *B. costata* wood, the dwindling use of the trees for timber resources has become a problem that can hardly be ignored. 25 to 32 % of the total wood volume belongs to branches, which are usually thrown away (Dadzie *et al*. 2016). Therefore, exploring the utilization of *B. costata* branchwood appears to be one immediate alternative and resource supplement.

Considering the above reasons, the aims of this study were to determine the wood anatomical features of *B. costata*, compare the anatomical features from two different sites in China, and investigate variations in the anatomical features of branchwood and stemwood, and heartwood and sapwood.

EXPERIMENTAL

Materials

Six mature trees were used in this study. Three of each were sampled from two different locations, Muzhaling Mountain in Henan Province, central China (111°24’–°32’E, 33°35’–40’N, 1750-melevation) and the Maoershan Forest Ecosystem Research Station in Heilongjiang Province, northeastern China (127°30’–34’E, 45°20’–25’N, 300melevation), which are both within the natural geographical distribution of *B. costata* (Chen *et al*. 2000). The Muzhaling site has an average slope of 10° to15°. The soil is a brown, neutral, sandy clay. The climate is a continental monsoon type with a mean annual precipitation of 700 mm, evaporation of 1600 mm, and air temperature of 14 °C, and 236-d frost-free period (Chen *et al*. 2016). The Maoershan site has an average slope of 20° to

25°. The soil is dark brown with abundant organic matter and a high fertility. The climate is a continental monsoon type with a mean annual precipitation of 700 mm, evaporation of 884 mm, air temperature of 2.8 °C, and 130-d frost-free period (Zhao 2015).

These sample trees ranged from 17 m to 24 m in height and 18.4 cm to 23.1 cm in diameter at breast height (1.3 m). From the middle canopy each sample tree, one branch was chosen to conform to the specifications as a standard branch, which was based on the average length and diameter (20 cm above the basal collar) of all branches. Branches often bend at the branch collar, where reaction wood is prone to occur (Groover 2016; Kidombo and Dean 2018). Thus, the diameter of the branch was measured about 20 cm above the basal collar. The standard branch diameters ranged from 8 cm to 12 cm. The selected trees and branches were somewhat smaller than average, but a considerable amount of mature wood was included in them. Five-centimeter-thick disc samples were taken from the branch and the stem at breast height. The disc from the branch was sampled about 20 cm above the basal collar in order to avoid any abnormality. It is easy to separate *B. costata* heartwood from sapwood with the naked eye because of color difference (Luo *et al*. 2012). A 1.5-cm wide strip (from pith to bark) was sawed from each disc. The strip was chosen from the upper part of the branch transverse section. The strip was then separated equally into two small strips, which were then used in wood sectioning and the macerating process.

Methods

Anatomical features measurement

The small strips used for wood sectioning were softened by soaking them in a solution of 5% ethylenediamine. A15-µm-thick transverse section, tangential section, and radial section were made from each small strip using a Leica slicing machine (Leica RM2235, Leica microsystem AG, Wetzlar, Germany), stained with 1% safranin in water, and then placed on microscopic slides for measurement (Zhao 2015).

The small strips for the maceration process were divided into small chips from heartwood and sapwood respectively, using a razor blade. Each chip was placed in an individual test tube and macerated with a 1:1 10% chromic acid:10% nitric acid solution (Jeffrey 1917). The test tubes were then placed in a water bath at 60 °C for several hours to hasten the maceration process. The macerated material was rinsed and placed on microscopic slides for measurement. Digital images of the microscopic slides were taken using a digital microscope (Mshot-MD50, Micro-shot Technology Limited, Guangzhou, China). Proportions of the wood elements (fiber, vessel, and ray), the tangential and radial diameters of the vessel in the transverse section, and the fiber size were measured with an image computer analysis system (TDY-5.2, Beijing Tian Di Yu Technology Co. Ltd., Beijing, China), as was previously described by Yu *et al*. (2009). At least 60 measurements were performed for each sample per parameter.

Statistical analysis

Differences between the two sites (Muzhaling and Maoershan), between the branchwood and stemwood, and between the heartwood and sapwood were evaluated by analyses of variance (ANOVA), followed by separation of the means by the least significant difference. An ANOVA test and multiple comparisons were performed using SPSS Statistics software (Version 24.0, International Business Machines Corporation, Armonk, New York, United States) with the significance assessed at a p less than 0.05.

RESULTS AND DISCUSSION

Wood Anatomy of *B. costata*

The analysis of the anatomical features of the *B. costata* wood showed that the vessels were either arranged as radial multiples or were solitary (Fig. 1). As reported by previous studies (Guo *et al*. 1996), solitary vessels were more frequent than radial multiples in the stemwood (Fig. 1a). However, radial multiples were more frequent than solitary vessels in the branchwood (Fig. 1b). The vessels had strictly alternate intervessel pitting and scalariform perforation plates in all of the stemwood and branchwood samples (Fig. 1c). No tyloses were found in the vessels, which suggested that *B. costata* wood was not very resistant to preservative treatments (DeMicco *et al*. 2016). The longitudinal parenchyma cells were not abundant in the stemwood, and they were even more scarce in the branchwood. The types of rays were multiseriate (2 to 4 seriates) with an accretion of 9 cells to 40 cells and uniseriate with an accretion of 4 cells to 15 cells (Fig. 1d). The gums were often contained in the ray cells. The rays were mostly homogeneous, with procumbent ray parenchyma cells and numerous cross-field pitting.

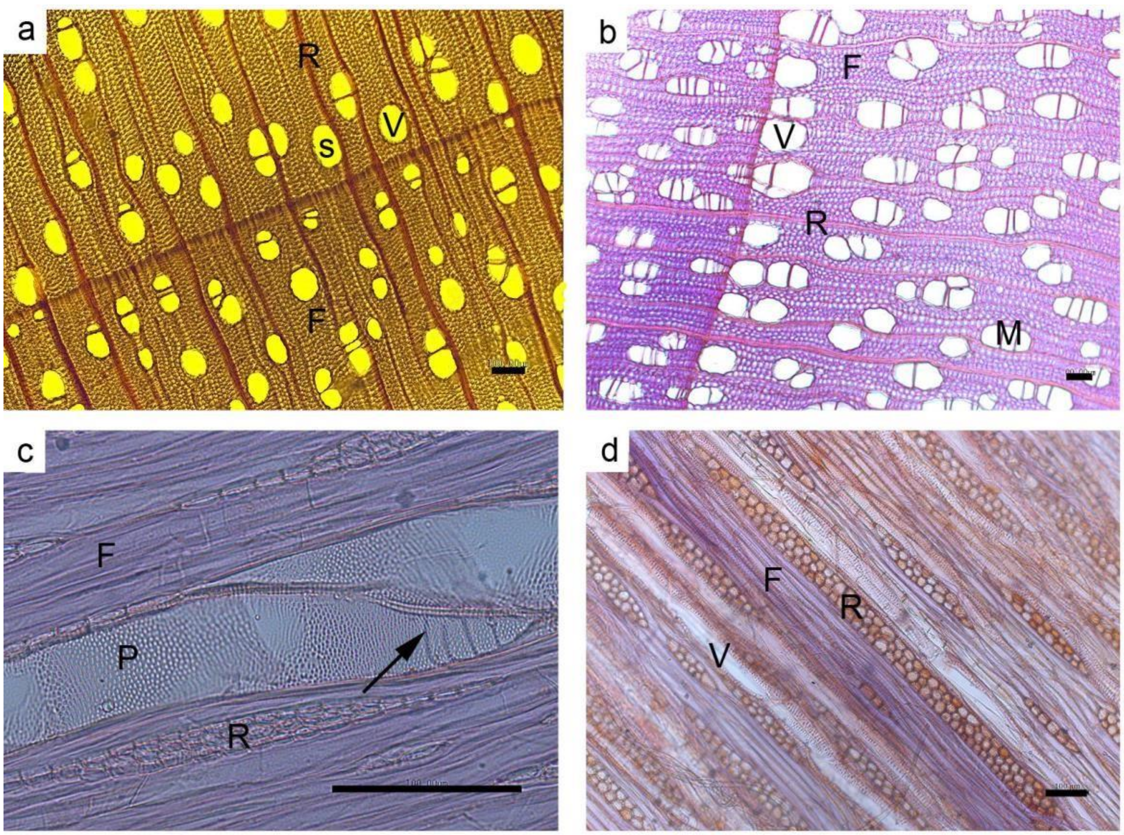

Fig. 1. Xylem sections displaying anatomical features in the stem and branch of *B. costata* Trautv. a:transverse section of the stemwood; b: transverse section of the branchwood; c: intervessel pitting and scalariform perforation plates in tangential section; d: types of rays in tangential section; F: fibre; V: vessel element; R: ray; M: multiple vessels; S: solitary vessel; P: intervessel pitting; Vessel elements come in series scalariform perforation plates (marked by arrows); scale bar is 100 μm.

Tissue Proportions

The results of the present study confirmed that the differences in the wood element proportions between the two different sites were not statistically significant, except for the fiber proportion. Lengthening of the frost-free period increases the growing season available for growth and the amount of xylem production (Sergio *et al*. 2014). The frost-free period in Muzhaling is longer than that in Maoershan (see Materials section), which may be one of reasons that the fiber proportion of *B.costata* from Muzaling significantly higher than that of Maoershan. The differences between the stemwood and branchwood were significant at the 5% level, except for the ray proportion (Table 1).

Table 1. ANOVA for the Anatomical Features in the Stemwood and Branchwood from *B. costata*

Anatomical Features	Sites	Stemwood and Branchwood	Sapwood and Heartwood
Fiber Proportion (%)	4.83*	5.24*	0.47
Vessel Proportion (%)	0.01	7.30*	3.61
Ray Proportion (%)	2.80	0.01	0.46
Fiber Length (μm)	11.64**	2.31	17.25**
Fiber Width (μm)	1.84	0.14	1.89
Fiber lumen Diameter (μm)	7.09**	0.37	1.10
Wall/Lumen	6.38*	1.89	0.07
Length/Width	10.16**	2.22	25.32**
Vessel Density (mm^{-2})	9.28*	8.38*	0.35
Vessel Tangential Diameter (μm)	1.36	83.76**	0.17
Vessel Radial Diameter (μm)	2.91	76.09**	0.06

*Significant at p<0.05, **Significant at p<0.01.

The fiber proportion in the *B. costata* wood was less than 40%, except in the sapwood of branch from the Muzhaling site (Table 2). These results indicated higher fiber yields for pulp from *B. costata* branchwood from Muzhaling. In general, if the fiber proportion of wood is higher, then the wood can be used as a raw material for papermaking (Stokke and Manwiller 1994). Thus, it was determined that it is one of advantageous factor for *B.costata* branchwood from Muzhaling to be used as a high-quality raw material for papermaking.

Table 2. Multiple Comparison of the Tissue Proportions in the Stemwood and Branchwood from *B. Costata* (%)

Site	Position	Fiber Proportion	Vessel Proportion	Ray Proportion
Muzhaling	Heartwood in branch	$35.5±0.8^{ab}$	$23.2±4.7^{ab}$	$41.6±5.5^{a}$
	Sapwood in branch	$43.7±5.9^{a}$	$19.0±2.9^{ab}$	$37.3±3.0^{a}$
	Heartwood in stem	$27.1±10.2^{b}$	$32.2±8.1^{a}$	$40.8±18.2^{a}$
	Sapwood in stem	$27.1±0.0^{b}$	$21.2±7.8^{ab}$	$51.8±7.8^{a}$
Maoershan	Heartwood in branch	$24.7±3.9^{b}$	$19.8±7.4^{ab}$	$55.5±11.2^{a}$
	Sapwood in branch	$29.9±3.7^{b}$	$17.8±1.4^{b}$	$52.2±2.4^{a}$
	Heartwood in stem	$25.8±6.7^{b}$	$32.1±4.0^{a}$	$42.1±10.7^{a}$
	Sapwood in stem	$22.7±2.2^{b}$	$25.3±4.8^{ab}$	$52.0±7.0^{a}$

Means±standard deviation with different letters within a column were significant at p<0.05

For *B. costata* wood from Maoershan and stemwood from Muzhaling, the lower fiber proportion implied that there was a higher vessel elements amount and rays proportion, which meant these woods, may had a lower density and strength and therefore be used for light construction purposes (Adeniyi *et al*. 2013).

There were differences in the tissue proportions between the sapwood and heartwood (Table 2). Transformation from sapwood to heartwood is a biological process within trees and has been related to individual tree biometric features, growth rates, site conditions, and genetic control (Pinto *et al*. 2004). The chemical components in the wood cell walls and extractives change during heartwood formation. The differences between heartwood and sapwood are important to both the solid-wood and pulp industries because the transformation from sapwood to heartwood changes the wood properties (Woeste 2002; Morais and Pereira 2007). Heartwood is valuable because of its color, scent, decay resistance, and other important properties. Because of poor resistance to fungal and insect attack, sapwood is classified as a defect region in wood in some factories (DeBell and Lachenbruch 2009). However, the differences in anatomical features between the heartwood and sapwood found in this study were not statistically significant at the 5% level (Table 1), which suggested that it is likely that sapwood can be engineered into a number of wood products.

Fibers

Statistically significant differences were found for the fiber length and lumen diameter, but not for the fiber width and double cell wall thickness between the selected sites. *Betula costata* from Maoershan had average significantly larger fiber sizes and smaller lumen than from Muzhaling (Table 3), which might be related to soil of growth sites. *B.costata* tends to grow well in fertile soil (Lee *et al*. 2004). The soil is abundant organic matter and high fertility in Maoershan but sandy clay in Muzhaling, which implies that large fibers are easier to be produced in *B. costata* from Maoershan than those from Muzhaling. The fiber sizes have an important effect on the final wood products. The average fiber length of *B. costata* was from 919 mm to 1628 mm, which met the intermediate standard for fibers (910 mm to1600 mm) stipulated by the International Society of Wood Anatomy (Smook 1992).The length/width ratio was also large, which indicated that *B. costata* wood is an excellent fiber material (Przybysz *et al*. 2018). Because of the large fiber size and small lumen, wood from *B. costata* trees from Maoershan could be more suitable for papermaking and glued plates than the trees from Muzhaling (Wang *et al*. 2009). Statistically significant differences in the fiber features were not found between the branchwood and stemwood from the *B. costata* trees, either at Maoershan or Muzhaling (Table 1). This indicated that branchwood might be an alternative to conventional stemwood from *B. costata* trees from Maoershan for use as a raw material in the wood and paper industry (Ni 1985).

Differences were found in the fiber features between the heartwood and sapwood of the *B.costata* (Table 3). Radial variation of the fiber features is determined by the cambial age (Lenz *et al*. 2010). A general pattern for the radial variation that has been recognized for most tree species is that the fiber features increase with the cambial age or distance from the pith to bark (Longui *et al*. 2014; Zhao 2015; Zhao *et al*. 2018). Aging and maturation cause genetically-controlled metabolic changes, and these lead to transformation from sapwood to heartwood in the center of the stem and branch (Wang *et al*. 2010).

Table 3. Multiple Comparison of the Fiber Features in the Stemwood and Branchwood from *B. costata*

Site	Position	Fiber Length (µm)	Fiber Width (µm)	Lumen Diameter (µm)	Double Wall Thickness	Wall/ Lumen	Length/ Width
Muzhaling	Heartwood in branch	919.2±391.7[c]	22.4±6.5[bc]	14.0±4.3[cd]	(8.5±3.5)[ab]	0.6±0.3[ab]	38.7±12.7[d]
	Sapwood in branch	1029.8±697.4[bc]	18.5±3.5[c]	11.7±2.9[d]	(6.8±1.3)[b]	0.6±0.2[abc]	56.2±37.3[bc]
	Heartwood in stem	1066.1±352.9[b]	23.6±4.5[b]	13.5±3.6[d]	(10.3±3.7)[ab]	0.9±0.5[a]	45.4±11.1[bcd]
	Sapwood in stem	1142.8±605.6[b]	25.5±7.3[ab]	17.9±6.4[bc]	(7.6±4.2)[ab]	0.5±0.3[bc]	44.2±19.7[cd]
Maoer-shan	Heartwood in branch	1289.1±506.7[ab]	29.3±6.3[a]	22.2±5.8[a]	(7.2±2.9)[ab]	0.4±0.2[cd]	44.4±16.8[cd]
	Sapwood in branch	1506.3±675.0[a]	25.0±4.7[ab]	22.2±5.3[a]	(4.9±1.4)[b]	0.2±0.1[d]	60.2±25.8[b]
	Heartwood in stem	976.4±170.5[bc]	25.6±5.4[ab]	19.0±4.7[ab]	(6.7±3.4)[b]	0.4±0.2[cd]	39.3±8.9[d]
	Sapwood in stem	1628.2±317.0[a]	22.7±4.7[bc]	14.2±4.0[cd]	(8.5±4.8) [b]	0.7±0.4[ab]	74.7±22.4[a]

Means ± standard deviation with different letters within a column were significant at p<0.05

Over the years, heartwood has been the main tree source in the wood industry because of its excellent characteristics. However, heartwood in the inner part and sapwood at the periphery of *B. costata* were found to be similar in fiber characteristics, which implied that sapwood use could widen the raw material base for the wood industry. The fibers of the sapwood were longer than that of the heartwood (Table 3). The average length and length/width of the fibers statistically differed between the heartwood and sapwood (Table 1). Long fibers are more flexible and conformable than short fibers (Ek *et al*. 2009). The fiber features of the sapwood and heartwood along the stems and branches of *B. costata* could provide adequate information to enhance its efficient utilization.

Vessels

Betula costata grown in Maoershan was found to have a significantly higher vessel density and lower vessel diameter than that from Muzhaling (Tables 1and 4), probably because there were large environmental differences between the sites (Hacke *et al*. 2017). The average evaporation and air temperature in Maoershan were 884 mm and 2.8 °C, which were far below those in Muzhaling. Trees in drier and colder sites have smaller vessels and a higher vessel frequency than trees in wetter and hotter sites (Dayer *et al*. 2017). Smaller vessels can be expected to be in less danger of catastrophic embolism and cavitation (Tyree and Sperry 1989) and are thus desirable under conditions with a high water stress. Larger vessels are more effective at conducting water to ensure hydraulic safety. However, vessels are particularly undesirable in wood pulp as it is easier for large vessels to cause picking, linting, and dusting problems during printing (Rakkolainen *et al*. 2009). The presence of vessels in wood also affects fluid penetrability and wood compression (Petty 1981; Sun *et al*. 2010). The results for the *B. costata*wood from Muzhaling suggested that its larger vessel diameter improves its suitability for processing liquids.

Table 4. Multiple Comparison of the Vessel Features in the Stemwood and Branchwood from *B. costata*

Site	Position	Vessel Density (mm^{-2})	Tangential Diameter (μm)	Radial Diameter (μm)
Muzhaling	Heartwood in branch	78.7±7.7^{bc}	163.7±33.0^{b}	164.8±57.9^{c}
	Sapwood in branch	60.6±12.8^{bc}	173.6±49.7^{b}	158.7±47.5^{c}
	Heartwood in stem	47.4±12.1^{bc}	252.5±55.3^{a}	281.4±116.2^{ab}
	Sapwood in stem	28.7±13.2^{c}	248.4±74.8^{a}	311.8±93.3^{a}
Maoershan	Heartwood in branch	139.2±1.8^{a}	139.2±43.9^{b}	136.2±42.3^{c}
	Sapwood in branch	148.1±6.3^{a}	145.9±32.3^{b}	137.4±47.5^{c}
	Heartwood in stem	83.8±50.9^{b}	262.9±67.6^{a}	280.1±115.8^{ab}
	Sapwood in stem	58.8±17.7^{bc}	228.8±68.1^{a}	235.9±73.4^{b}

Means±standard deviation with different letters within a column were significant at $p<0.05$

Insignificant variation in the vessel features between the heartwood and sapwood was observed, whereas the variation of stemwood and branchwood showed significant differences in the vessel features (Table 1). The average vessel features of the branchwood showed a higher vessel density and lower diameters than those of the stemwood on both sites (Table 4). The axial variation in the vessel lumen diameter and vessel density of the *B. costata* was consistent with the optimum water transport network of wide conduits at the stem base, which feed an increasing number of narrower branch conduits distally (McCulloh *et al.* 2003). The pattern of vessel tapering and density increasing from the stem to branch can maximize hydraulic conductivity and minimize xylem vulnerability (Pittermann and Olson 2018). The size of the vessels in stemwood is largely beneficial for processing preservatives, water-soluble dye, or other permeable liquids. Compared with stemwood, vessel tapering in branchwood increases the dimensional stability of lumber and can also reduce vessel picking problems in papermaking (Rakkolainen *et al.* 2009).

CONCLUSIONS

1. Anatomic differences of the *B. costata* wood between the two sites, between the branchwood and stemwood, and between the sapwood and heartwood were demonstrated across all monitored parameters. This can be attributed to site environment, wood age, as well as function of wood cells.
2. The *B. costata* branchwood from Maoershan might be suitable for papermaking and glued plates because of its intermediate fiber length and other suitable anatomical features. The *B. costata* stemwood from Muzhaling and the *B. costata* wood from Maoershan might be used for light construction purposes.
3. Observation was comprehensive for only anatomical features of *B. costata* wood. Since this does not constitute a comprehensive study of wood properties that

determining wood in utilization purposes, a more complete analysis of the chemical components, physical, and mechanical properties should be carried out in the future.

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