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## Fruit oil contents of the genus *Quercus* (Fagaceae): A comparative study on acorns of subgenus *Quercus* and the Asian subgenus *Cyclobalanopsis*

K. XIA<sup>1,2</sup>, C.E. SEAL<sup>3</sup>, W.-Y. CHEN<sup>1</sup>, Z.-K. ZHOU<sup>1\*</sup> AND H.W. PRITCHARD<sup>3</sup>

<sup>1</sup> Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming 650204, China (E-mail: zhouzk@mail.kib.ac.cn)

<sup>2</sup> Graduate University of Chinese Academy of Sciences, Beijing 100049, China

<sup>3</sup> Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place, RH17 6TN, United Kingdom

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### Summary

Seed oils are an essential energy reserve for germination and early seedling development for many species, yet quantification is still relatively rare beyond the main crops. The genus *Quercus* (Fagaceae), consisting of subgenera *Quercus* and *Cyclobalanopsis*, is widespread throughout the Northern Hemisphere; however, fruit / acorn oil content of the Asian distributed subgenus *Cyclobalanopsis* is not well documented. We quantified fruit oil contents in eight species from the subgenus *Cyclobalanopsis* (from China) and ten species from the subgenus *Quercus* (from both China and Europe) by supercritical fluid extraction with carbon dioxide. The majority of fruits were highly viable with a germination of over 50%. Fruit oil content of subgenus *Cyclobalanopsis* was significantly lower than that of subgenus *Quercus* and across 18 species studied moisture content of the storage tissue (cotyledons) was negatively related to fruit oil content. Our oil content data were combined with that from the literature, resulting in a total of 57 species, and mapped on the current phylogeny for *Quercus* to reveal the highest fruit oil contents associated with sect. *Lobatae*.

### Introduction

The genus *Quercus* consists of subgenera *Quercus* and *Cyclobalanopsis* and has approximately 450 species, making this the largest and most widely distributed genus within the Fabaceae family, occurring throughout temperate and subtropical montane areas of the Northern Hemisphere (Camus, 1936-1954; Nixon, 1993; Frodin and Govaerts, 1998; Huang *et al.*, 1999). Subgenus *Cyclobalanopsis* (with approximately 150 species) is predominately distributed across tropical and subtropical Asia and is a dominant tree in Asian evergreen broad-leaved forests (Nixon, 1993; Huang *et al.*, 1999, Luo and Zhou, 2001), with the greatest concentration of species in E and SE Asia, and the Indochinese Peninsula. In contrast, subgenus *Quercus* is widely distributed throughout the temperate regions of the Northern Hemisphere and is particularly rich in the temperate and semi-arid flora of North and Central America (Nixon, 1993 and 1997; Li, 1996). Subgenus

\* Author for correspondence

*Quercus* includes four sections (sect.): *Quercus* s. s. (white oaks) (Manos *et al.*, 1999), *Cerris* (Camus, 1936-1954), *Lobatae* (black/red oaks) and *Protobalanus* (golden cup or intermediate oaks) (Nixon, 1993 and 1997).

Seed oils are an essential energy reserve for germination and early seedling development for many species (Mayer and Poljakoff-Mayber, 1963) and are widely studied in the context of seed longevity and ageing due to their influence on water relations (Cromarty *et al.*, 1982; Benson, 1990; Probert, 2003; Pritchard and Dickie, 2003). In addition, seed oils have comestible value, including from *Quercus* fruits, for which there is evidence of a long history of people using them as a food source (Nixon, 1997).

Some information on fruit oil for subgenus *Quercus* can be found scattered throughout the literature; however, information of fruit oil for Asian species, especially subgenus *Cyclobalanopsis*, is lacking. To provide new data on fruit oil content of subgenus *Cyclobalanopsis* and to compare this with fruit oil content of subgenus *Quercus*, we investigated eight species from subgenus *Cyclobalanopsis* and three species from subgenus *Quercus*, collected across S and SW China, in addition to seven species of subgenus *Quercus* from Italy and Denmark (table 1). A review of the literature was also conducted, resulting in data for a total of 45 species of which 39 species were additional to our study, and used in conjunction with data from the 18 species in this study to examine the association of fruit oil with *Quercus* phylogeny.

## Materials and methods

Fruits were collected from the different locations (table 1) and stored in loosely tied polyethylene bags for less than two months in either a humidity room of 70% relative humidity (RH) at 15°C or in a 5°C incubator. Any cupules which were not detached during maturation were removed on the commencement of experiments. Germinability was assessed by testing a total of 25 fruits sown (in lots of 12 and 13 seeds) on 1% agar-water in Perspex sandwich boxes (175 × 115 × 60 mm) and incubated at 20°C with a photoperiod of 8 h/d (warm white fluorescent light at a photon flux density of 50-100 W/m<sup>2</sup>). Germination was determined as radicle emergence of > 2 mm and fruits were scored frequently for more than 6 months until no more germination was observed. At the end of the germination tests, the viability of any remaining fruits was checked by a cut test (ISTA, 2007).

Oil was extracted from fruits using supercritical fluid extraction with carbon dioxide (ISCO SFX 3560 fat analyser, ISCO inc., USA; with carbon dioxide C.P. grade, purity 99.995%). The experiment was carried out following the two-step extraction method described by Seal *et al.* (2008) on three fruits for each species. Fruits with high viability and showing no sign of infestation were selected for analysis. For the larger fruits, ground fruit material was placed into 2 or 3 extraction cartridges, analysed and a mean taken of these results to represent the whole fruit. The results of the oil content were calculated on a dry weight basis (dwt), where fruit moisture content was determined on five individual fruits gravimetrically by drying for 17 h at 103°C (ISTA, 2007). Differences in oil content were tested for significance using a One-Way ANOVA with Fisher's LSD *post hoc* analysis (Dytham, 2003).

Table 1. The taxa of the investigated *Quercus* species, location of the collection site and germination and moisture contents for the fruits studied. Germination was carried out on a total of 25 fruits (as lots of 12 and 13 seeds) and storage tissue (cotyledon) moisture contents on 5–25 individuals (mean ± SD).

Species (abbreviation)	Subgenus	Section	Location of Collection Site	Collecting time (month/year)	Percentage germination (mean±SE) (%)	Storage tissue moisture content before drying <sup>1</sup> (%)	Storage tissue moisture content after drying <sup>2</sup> (%)
<b>Chinese collection</b>							
<i>Q. annulata</i> (an)	<i>Cyclobalanopsis</i>	-	Guilin, Guangxi	10/06	76.0 ± 1.0	43.1 ± 6.1	9.6 ± 1.1
<i>Q. fleuryi</i> (fl)	<i>Cyclobalanopsis</i>	-	Guilin, Guangxi	11/07	80.6 ± 6.9	36.0 ± 4.7	6.8 ± 0.4
<i>Q. glauca</i> (gl)	<i>Cyclobalanopsis</i>	-	Hengshan Mountain, Human	11/06	87.8 ± 4.5	49.2 ± 4.0	11.4 ± 5.2
<i>Q. lamellosa</i> (la)	<i>Cyclobalanopsis</i>	-	Wuliang Mountain, Yunnan	10/06	30.0 ± 5.5	47.6 ± 7.3	13.6 ± 2.3
<i>Q. multinervis</i> (mu)	<i>Cyclobalanopsis</i>	-	Hengshan Mountain, Human	10/06	52.2 ± 6.1	53.4 ± 4.1	8.6 ± 0.8
<i>Q. schottekiana</i> (sc)	<i>Cyclobalanopsis</i>	-	Kunming, Yunnan	11/07	76.0 ± 1.0	46.3 ± 7.0	ND
<i>Q. sichourensis</i> (si)	<i>Cyclobalanopsis</i>	-	Funing, Yunnan	11/07	7.7 ± 4.5	46.6 ± 9.3	7.9 ± 0.8
<i>Q. stewardiana</i> (st)	<i>Cyclobalanopsis</i>	-	Lushan Mountain, Jiujiang, Jiangxi	10/06	44.2 ± 5.8	40.4 ± 3.2	8.7 ± 0.8
<i>Q. fabri</i> (fa)	<i>Quercus</i>	<i>Quercus</i>	Kunming, Yunnan	10/07	96.2 ± 3.8	42.4 ± 6.2	7.2 ± 0.6
<i>Q. franchetii</i> (fr)	<i>Quercus</i>	<i>Cerris</i>	Kunming, Yunnan	10/07	95.8 ± 4.2	44.2 ± 2.9	ND
<i>Q. variabilis</i> (va)	<i>Quercus</i>	<i>Cerris</i>	Kunming, Yunnan	10/07	77.6 ± 13.3	43.4 ± 9.3	12.8 ± 2.4
<b>European collection</b>							
<i>Q. petraea</i> (pe)	<i>Quercus</i>	<i>Quercus</i>	Kirstinebjergvej, Årslev, Denmark	12/06	92.0 ± 0.3	41.9 ± 3.4	7.9 ± 1.1
<i>Q. pubescens</i> (pu)	<i>Quercus</i>	<i>Quercus</i>	de Fasse-Pissoiotto Verona, Italy	11/06	23.7 ± 7.1	36.9 ± 1.8	8.6 ± 0.8
<i>Q. robur</i> (ro)	<i>Quercus</i>	<i>Quercus</i>	Bosco della Partecipiano, Novara, Vercelli, Italy	11/06	72.1 ± 2.9	36.0 ± 1.5	ND
<i>Q. cerris</i> (ce)	<i>Quercus</i>	<i>Cerris</i>	Rolasco, Alessandria, Italy	11/06	92.0 ± 0.3	35.6 ± 2.1	10.7 ± 1.1
<i>Q. ilex</i> (il)	<i>Quercus</i>	<i>Cerris</i>	Bosco della Tesolo, Ferrara, Italy	11/06	72.4 ± 10.9	39.2 ± 3.6	7.9 ± 0.4
<i>Q. suber</i> (su)	<i>Quercus</i>	<i>Cerris</i>	Ducua Fecuglio, Grosseto, Italy	11/06	72.1 ± 0.6	52.3 ± 3.7	9.0 ± 1.6
<i>Q. rubra</i> (ru)	<i>Quercus</i>	<i>Lobatae</i>	R.N.O. Boscofontana, Milano, Italy	11/06	56.7 ± 18.3	28.6 ± 2.3	6.0 ± 0.5

<sup>1</sup> Seed RH varied from 83 to 98%; <sup>2</sup> Seed RH ranged from 10–16%. ND, not determined; in the time course of this experiment *Quercus robur*, *Q. franchetii* and *Q. schottekiana* did not reach this low RH.

Fruit RH and storage tissue (cotyledon) moisture contents were determined before and after drying. RH was determined thrice after 30 min at c. 20°C in a Rotronic HygroPalm water activity monitor fitted with a AWVC-D10 sensor (Rotronic Ltd., UK). Moisture contents of the cotyledons were determined on 25 (pre-dried) or 5 (dried) individual fruits after oven drying at 103°C for 17 h (ISTA, 2007) and are reported on a fresh weight (%) basis. For drying, fruits were sealed in plastic bags (regularly vented) with freshly-regenerated silica gel at a weight ratio of 1:1 and held at 15°C. The fresh weight of the fruits was monitored every day and the target RH of c. 15% (i.e. international seed bank standard) was achieved in the time course of the experiment for 15 of the 18 species studied.

## Results and discussion

The majority of species were highly viable with a final germination of more than 50% (table 1). Cut tests carried out on non-germinated fruits showed that infestation by fungus and weevils resulted in the death of the fruits (data not shown).

The fruit oil content for the genus *Quercus* ranged from 0.7% for *Q. lamellosa* Sm. to 18.0% for *Q. rubra* L. (figure 1). Fruits of subgenus *Cyclobalanopsis* had oil contents between 0.7% and 3.8% and were significantly lower ( $p < 0.001$ ) than that of subgenus *Quercus* which ranged from 1.5% to 18.0%. Within subgenus *Quercus*, there was no significant difference in fruit oil content between the species from sect. *Quercus* and sect. *Cerris*. Fruits of *Q. rubra* (sect. *Lobatae*) were high in oil (figure 1) in agreement with previous studies that found higher oil in red and black oaks (sect. *Lobatae*) than in white acorns (sect. *Quercus*) (Wainio and Forbes, 1941; Short, 1976; Short and Epps, 1976; Vander Wall, 2001).

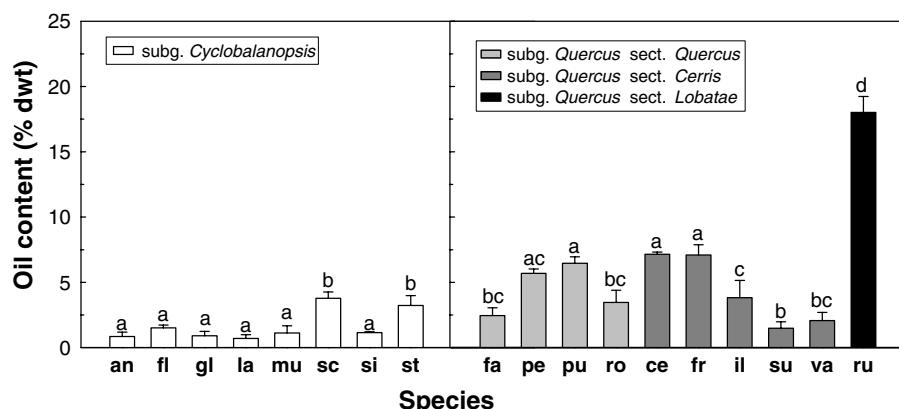


Figure 1. The oil content (% dwt) in fruits of genus *Quercus* subgenus *Cyclobalanopsis* (left, white bars) and subgenus *Quercus* (right). The species from subgenus *Quercus* were grouped by sections *Quercus* (grey bars), *Cerris* (dark grey bars) and *Lobatae* (black bar). See table 1 for full name of abbreviations. Values with the same letters within the same subgenus were not significantly different at  $p > 0.05$  (One-Way ANOVA with Fisher's LSD post hoc analysis). Data are the means of three replicates  $\pm$  SE.

The moisture content of the storage tissue (cotyledons) was negatively related to fruit oil content over the range 1 – 18% (figure 2), with the dependency significant for the moister seeds at c. 90% RH ( $p = 0.001$ ). The reduction in seed (and tissue) moisture content with increasing oil content has been widely reported, particularly for crop seeds (Cromarty *et al.*, 1982; Pritchard and Dickie, 2003; Probert, 2003). Therefore, in freshly harvested fruits of *Quercus* (and seeds of other species) differences in moisture contents between species does not necessarily mean variance in water relations *per se*; consideration should also be given to possible influence of variation in seed / tissue oil content.

Based on our results (figure 1) and the data retrieved from the literature (table 2), the oil contents in acorns of genus *Quercus* were mapped against the recent phylogeny described by Oh and Manos (2008) (figure 3). The results of this combined summary support our findings of low oil contents (0.7-3.8%) in subgenus *Cyclobalanopsis*, and lower than all the sections in subgenus *Quercus*.

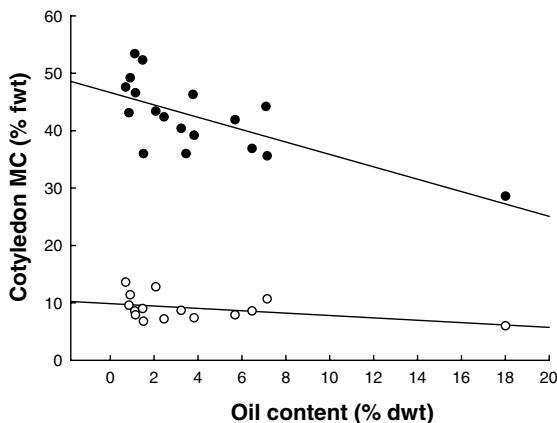


Figure 2. Dependency of cotyledon (storage tissue) moisture content (MC) on oil content for *Quercus* species. Fruits were at 83-98% RH and 10-16% RH before and after drying, respectively. Fitted line parameters are:  $y = -1.078x + 46.64$ ,  $R^2 = 0.49$ ,  $p = 0.001$  (●) and  $y = -0.21x + 9.86$ ,  $R^2 = 0.18$ ,  $p = 0.12$  (○). Note that the data are drawn from figure 1 and table 1 and include 18 and 15 species for the pre-dried and dried treatments respectively, as the fruits of three species (*Quercus robur*, *Q. franchetii* and *Q. schottkyana*) did not reach low RH.

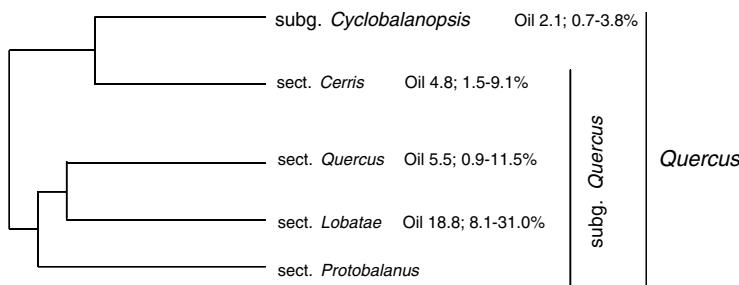


Figure 3. The oil contents (% dwt) of acorns from the genus *Quercus* based on the phylogenetic relationship of Oh and Manos (2008), showed as the mean value and range of oil content of each subgenus and section (mean; range).

Table 2. The oil content of *Quercus* acorns on a percentage dry-weight basis (% dwt). Data includes both fruits with and without the pericarp (which does not alter the statistical significance of the results).

Subgenus	Section	Species	Location of Collection Site	Mean Oil Content <sup>a</sup> (% dwt)	Source
<i>Cyclobalanopsis</i>		<i>Q. glilva</i>	Japan	3.4	Matsuyama (1982); Shimada and Saitoh (2006)
		<i>Q. glauca</i>	China, Japan	2.6	Matsuyama (1982); Shimada and Saitoh (2006); Xiao <i>et al.</i> (2006)
		<i>Q. myrsinæfolia</i>	Japan	3.4	Matsuyama (1982); Shimada and Saitoh (2006)
<i>Quercus</i>	<i>Quercus</i>	<i>Q. alba</i>	N. America	5.6	Wainio and Forbes (1941); Baungras (1944); Gysel (1957); Bonner (1971, 1974); Smith and Follmer (1972); Short (1976); Servello and Kirpatrick (1989)
		<i>Q. aliena</i>	Japan	2.8	Shimada and Saitoh (2006)
		<i>Q. chapmanii</i>	N. America	4.1	Abrahamson and Abrahamson (1989)
		<i>Q. crispula</i> (syn. <i>Q. mongolica</i> var. <i>grosseserrata</i> )	Japan	1.7	Shimada (2001b)
		<i>Q. douglasii</i>	N. America	8.3	Koenig and Mumme (1987)
		<i>Q. germinata</i>	N. America	4.7	Abrahamson and Abrahamson (1989)
		<i>Q. lobata</i>	N. America	7.7	Koenig and Mumme (1987); Koenig (1991); Koenig and Faeth (1998)
		<i>Q. lyrata</i>	N. America	1.8	Bonner (1971, 1974); Ofcarcik and Burns (1971)
		<i>Q. macrocarpa</i>	N. America	7.8	Bonner (1971, 1974); Ofcarcik and Burns (1971); Smith and Follmer (1972); Briggs and Smith (1989)
		<i>Q. michauxii</i>	N. America	4.0	Bonner (1971, 1974); Ofcarcik and Burns (1971)
		<i>Q. minima</i>	N. America	4.7	Abrahamson and Abrahamson (1989)
		<i>Q. muehlenbergii</i>	N. America	5.7	Bonner (1971, 1974); Briggs and Smith (1989)

<sup>a</sup>across published studies

Table 2. *continued*

Subgenus	Section	Species	Location of Collection Site	Mean Oil Content <sup>1</sup> (% dwt)	Source
<i>Quercus</i>	<i>Quercus</i>	<i>Q. petraea</i> (syn. <i>Q. sessilis</i> )	Poland, Switzerland	5.9	Drozd (1968); Shimada and Saitoh (2006)
		<i>Q. prinoides</i>	N. America	6.3	Wainio and Forbes (1941)
		<i>Q. pyrenaica</i>	Portugal	3.8	Ferreia-Dias <i>et al.</i> (2003)
		<i>Q. robur</i>	Switzerland	4.9	Shimada and Saitoh (2006)
		<i>Q. serrata</i>	Japan, China	2.8	Shimada (2001a,b); Xiao <i>et al.</i> (2006)
		<i>Q. sinuata</i> (syn. <i>Q. durandii</i> )	N. America	3.8	Bonner (1971, 1974)
		<i>Q. stellata</i>	N. America	6.7	Bonner (1971, 1974); Ofarcik and Burns (1971); Short (1976); Briggs and Smith (1989)
		<i>Q. virginiana</i>	N. America	7.9	Bonner (1971, 1974); Ofarcik and Burns (1971); Short (1976); Wainio and Forbes (1941); Short (1976); Smallwood and Peters (1986); Servello and Kirpatrick (1989)
		<i>Q. montana</i> (syn. <i>Q. prinus</i> )	N. America	6.7	
		<i>Q. acutissima</i>	Japan, N. America	4.3	Short (1976); Matsuyama (1982); Shimada and Saitoh (2006)
<i>Cerris</i>	<i>Q. rotundifolia</i>		Portugal	9.1	Ferreia-Dias <i>et al.</i> (2003)
			Portugal	5.2	Ferreia-Dias <i>et al.</i> (2003)
		<i>Q. suber</i>			
		<i>Q. variabilis</i>	Japan, China	4.6	Shimada and Saitoh (2006); Xiao <i>et al.</i> (2006)
		<i>Q. agrifolia</i>	N. America	20.6	Koenig and Mumme (1987); Koenig (1991); Koenig and Faeth (1998)
		<i>Q. coccinea</i>	N. America	14.6	Bonner (1971, 1974)
<i>Lobatae</i>	<i>Q. falcata</i> (syn. <i>Q. falcata</i> var. <i>falcata</i> )	N. America	23.6	Bonner (1971, 1974); Ofarcik and Burns (1971); Short (1976)	

<sup>1</sup>across published studies

Table 2. *continued*

Subgenus	Section	Species	Location of Collection Site	Mean Oil Content <sup>1</sup> (% dwt)	Source
<i>Quercus</i>	<i>Lobatae</i>	<i>Q. ilicifolia</i>	N. America	20.0	Wainio and Forbes (1941)
		<i>Q. incana</i>	N. America	23.1	Ofcaričk and Burns (1971); Short (1976)
		<i>Q. inopina</i>	N. America	24.8	Abrahamson and Abrahamson (1989)
		<i>Q. laevis</i>	N. America	8.1	Abrahamson and Abrahamson (1989)
		<i>Q. marilandica</i>	N. America	16.9	Ofcaričk and Burns (1971)
		<i>Q. myrtifolia</i>	N. America	26.7	Abrahamson and Abrahamson (1989)
		<i>Q. nigra</i>	N. America	18.0	Ofcaričk and Burns (1971); Bonner (1971, 1974); Short (1976)
		<i>Q. pagoda</i> (syn. <i>Q. falcata</i> ) N. America var. <i>pagodaeifolia</i>		15.8	Bonner (1971, 1974)
		<i>Q. palustris</i>	N. America	13.6	Bonner (1971, 1974); Briggs and Smith (1989)
		<i>Q. phellos</i>	N. America	16.9	Bonner (1971, 1974); Ofcaričk and Burns (1971); Short (1976)
		<i>Q. rubra</i>	N. America	19.1	Wainio and Forbes (1941); Baumgras (1944); Gysel (1951); Short (1976); Briggs and Smith (1989); Servello and Kirpatrick (1989)
		<i>Q. shumardii</i>	N. America	18.1	Bonner (1971, 1974); Ofcaričk and Burns (1971); Smith and Follmer (1972)
		<i>Q. texana</i> (syn. <i>Q. nuttallii</i> )	N. America	13.2	Bonner (1971, 1974)
		<i>Q. velutina</i>	N. America	19.4	Baumgras (1944); Gysel (1957); Short (1976); Briggs and Smith (1989)

<sup>1</sup>across published studies

There were also no significant differences in oil content between sect. *Quercus* and sect. *Cerris*. Moreover, sect. *Lobatae* had the highest oil (8.1-31.0%), suggesting the evolution of elevated oil content in this section is a more recent event in the divergence of subgenus *Quercus*.

In summary, the 57 species from our experimental data and the published literature, revealed interesting phylogenetic patterns in fruit / acorn oil content within the genus *Quercus*, including low oil contents associated with the previously uninvestigated Asian subgenus *Cyclobalanopsis*.

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