GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Research / Araştırma
GIDA (2022) 47 (2) 212-219
doi: 10.15237/gida.GD21147

COMPARISON OF POLLEN MORPHOLOGIES EXAMINATION OF SOME *Rhododendron* SPECIES - PLANT SOURCE OF MAD HONEY

Aslı Özkök[1,2], Nazlı Mayda[3], Nesrin Ecem Bayram[4,*]

[1]Department of Biology, Faculty of Sciences, Hacettepe University, Ankara, Turkey
[2]Hacettepe University, Bee and Bee Products Application and Research Center, Ankara, Turkey
[3]Department of Pharmaceutical Botany, Pharmacy Faculty, Trakya University, Edirne, Turkey
[4]Department of Food Processing, Aydıntepe Vocational College, Bayburt University, Bayburt, Turkey

Received / Geliş: 03.12.2021; *Accepted* / Kabul: 27.01.2022; *Published online* / Online baskı: 05.03.2022

Özkök, A., Mayda, N., Ecem-Bayram, N. (2022). Comparison of pollen morphologies examination of some Rhododendron species - plant source of mad honey. GIDA (2022) 47 (2) 212-219 doi: 10.15237/gida.GD21147.

Özkök, A., Mayda, N., Ecem-Bayram, N. (2022). Deli bal'ın kaynağı olan *Rhododendron* türlerinin polen morfolojilerinin karşılaştırılması. *GIDA* (2022) 47 (2) 212-219 doi: 10.15237/gida.GD21147.

ABSTRACT

Honey is one of the important natural products used for apitherapeutic purposes as well as food consumption. The authenticity of the products to be used for apitherapy is very important. Botanical origin is one of the most critical factors affecting the characterization of bee products, especially honey. Therefore, melissopalynological analysis is essential in honey samples. In this study, the pollen morphologies of four different *Rhododendron* taxa, the origin of the monofloral "Mad honey" used for apitherapeutic purposes in Turkey, were examined. Five different measurement parameters have been used to investigate the pollen morphology of the plants. As a result, it can be said that pollen morphology has an important place in confirming the botanical origin of "Mad honey".

Keywords: Palynology, pollen morphology, *Rhododendron* spp., mad honey.

DELI BAL'IN KAYNAĞI OLAN *Rhododendron* TÜRLERİNİN POLEN MORFOLOJİLERİNİN KARŞILAŞTIRILMASI

ÖZ

Bal, gıda olarak tüketiminin yanı sıra apiterapik amaçlarla da kullanılan önemli doğal ürünlerden birisidir. Apiterapide kullanılacak ürünlerin orijinalliği çok önemlidir. Botanik köken, başta bal olmak üzere arı ürünlerinin karakterizasyonunu etkileyen en önemli faktörlerden biridir. Bu noktada melissopalinolojik analizlerin önemi ortaya çıkmaktadır. Bu çalışmada, Türkiye'de apiterapik amaçlı kullanılan monofloral "Deli bal"a kaynaklık eden dört farklı *Rhododendron* taksonunun polen morfolojisi incelenmiştir. Bitkilerin polen morfolojisinin araştırılmasında beş farklı ölçüm parametresi kullanılmıştır. Sonuç olarak, "Deli bal"ın botanik kökeninin doğrulanmasında polen morfolojisinin önemli bir yeri olduğu söylenebilir.

Anahtar kelimeler: Palinoloji, polen morfolojisi, *Rhododendron* spp., deli bal.

* *Corresponding author* / Yazışmalardan sorumlu yazar
: ecem.nesrin@gmail.com : (+90) 458 333 4426 : (+90) 458 333 4466

Aslı Özkök; ORCID no: 0000-0002-7336-2892
Nazlı Mayda; ORCID no: 0000-0002-7289-5830
Nesrin Ecem Bayram; ORCID no: 0000-0002-5496-8194

INTRODUCTION

Ericaceae is the 8 th largest family of Angiosperms. It is represented by about eight subfamilies, 125 genera and 4100 species in the world. *Rhododendron* L., one of the largest genus of this family, contains more than 1000 taxa in the world. More than 850 species are distributed in the Northern Hemisphere and can be found from sea level to 5000 m altitude (Chamberlain et al., 1996; Fang et al., 2005). *Rhododendron* taxa are mostly evergreen, rarely deciduous shrubs, semi-shrubs, trees, or sometimes climbing plants. The leaves are alternate, rarely opposite or circular, simple and without stipules. The leaves of plants that adapt to arid environments are reduced (Terzioğlu et al., 2001; Yıldız and Aktoklu, 2010). The inflorescence is usually terminal. The flowers are zygomorphic. Sepals free or united. The corolla is funnel-shaped. Anthers are connected externally and open by cleavage. The ovary has five loci, nectar is prominent. Stigma capitate. Fruit septicide capsule. Seeds are winged, testa cells are thin-walled (Stevens, 1978). *Rhododendron* genus is divided into 8 subgenus according to their morphological features; *Rhododendron* L., subgenus *Hymenanthes* (Blume) K.Koch, subgenus *Pentanthera* (G.Don), subgenus *Tsutsusi* (Sweet) Pojarkova, subgenus *Azaleastrum* (Planch), subgenus *Candidastrum* (Franch), subgenus *Mumeazalea* (Sleumer) Philipson & Philipson, subgenus *Therorhodion* (Maxim.) A. Gray (Cox and Cox, 1997). However, in the latest studies, all Turkish *Rhododendron* plants consist of 5 species and 12 taxa, four of which are hybrids (Anşin and Terzioğlu, 1994; Milne et al., 1999; Terzioğlu et al., 2001). These are *R. luteum* Sweet, *R. ungernii* Trautv., *R. smirnovii* Trautv., *R. caucasicum* Pallas, *R. ponticum* L. subsp. *ponticum* var. *ponticum*, *R. ponticum* L. subsp. *ponticum* var. *heterophyllum* Anşin, *R. ponticum* L. subsp. *ponticum* forma album (Sweet) Zab., *Rhododendron ponticum* L. subsp. *baeticum* (Boiss. & Reut.) Hand.-Mazz. (suspicious record (Davis, 1978)), *Rhododendron × sochadzeae* Charadze & Davlianidze, *Rhododendron × rosifaciens* R. Milne, *Rhododendron × davisianum* R. Milne, *Rhododendron × filidactylis* R. Milne. All Turkish taxa except for *R. luteum* are evergreen shrubs and generally distributed in the North, especially in the Eastern Black Sea from low montane forests to alpine regions more than 3000 m high (Davis, 1978; Terzioğlu et al., 2001; Avcı, 2004). *Rhododendron* species have an important place in honey production because they have a long flowering period, carry abundant flowers and have nectar (Çeter and Güney, 2011). Two members of this genus, contain *R. ponticum* and *R. luteum* are well known for being poisonous due to andromedotoxin component of the flower (Taşdemir et al., 2003). These species, particularly *R. ponticum*, are common folk medicines and are widely used as an analgesic to treat rheumatic or dental pain, common colds and edema in the eastern Black Sea Region of Turkey (Baytop, 1999). Also, the honey made up from these nectar plants, locally known as "mad honey" (deli bal in Turkish), has hypotensive properties and causes intoxications in humans (Onat et al., 1991; Sütlüpınar et al., 1993; Baytop, 1999). Because of this characteristic of mad honey, the determination of the origin of toxic and non-toxic species in honey can provide an important tool in separating and packaging honey and preventing unwanted threats to human health. The pollen morphology of *Rhododendron* species is generally well studied (Yang et al., 2003; Wang et al., 2006; Zhang et al., 2009); however, there is limited information regarding the species from North-East Turkey. Milne et al. (1999) investigated the hybridization between sympatric *Rhododendron* species distributed in Turkey at both the morphological and molecular levels. These researchers reported that it is not a coincidence that *Rhododendron* species are concentrated in the Eastern Black Sea region, where precipitation and temperature values differ in terms of climatic conditions. Morphological and molecular studies have revealed that ecological characteristics are essential in natural hybridization among *Rhododendron* species. Yan et al. (2014) conducted studies on biodiversity and endemism with a DNA barcoding system in the Himalaya-Hengduan mountains, which is the richest region in terms of plant diversity in China. It has been reported that there are difficulties in classifying the *Rhododendron* genus in this area because of its great diversity. For this reason, as predicted in this study, it is thought that the data presented for the classification of these plants that contribute to

honey are important. Therefore, in this study, we examined the interspecific variation of the pollen type of four Turkish species, which are flowering at the same time in the Ayder Plateau as one of the important beekeeping centers in North East Turkey.

MATERIAL AND METHODS

Plant samples (*R. luteum* (zifin in Turkish), *R. ponticum* (kumar in Turkish), *R. ungernii* (beyaz kumar in Turkish), *R. caucasicum* (dağ kumara in Turkish) were collected from the high plateaus (1800-3000 m) of Rize, Turkey on May 2017. Voucher specimens were deposited at the Herbarium of the Faculty of Science, Hacettepe University in Ankara.

Measurements of the pollen grains were taken on 50 pollen grains per species by a lens (X40) (Generally, pollen measurement is made by X100 object-lens. But in this study, we made it X40 because *Rhododendron* pollens are bigger). After that, pollen slides were prepared by Wodehose (1965) method from the plant anther. The plant anthers were crushed with a dissection needle on a slide and wet with ethanol (70%). After the ethanol evaporated, about 1 mm^3 basic fuchsin glycerin-gelatine matrix was added to it. That slide was put on a hot plate when gelatin melted and coverslip closed. Pollen slides were left to dry upside down for one night. Pollen tetrads were examined, and measurements were made under the light microscope Nikon Eclipse E400 and photographed with an XCAM Family 1080P HDMI camera. Minimum 30 pollen grains were examined (three times) for morphological investigation. Five parameters were investigated under LM. Those parameters; total length of pollen tetrads (Ml), width of a pollen in tetrad (lt), height of a pollen in tetrad (lg), semi- length of colpus on a pollen tetrad (f), one side of triangle polar on a pollen in tetrad (pt). That parameters are shown in Figure 1.

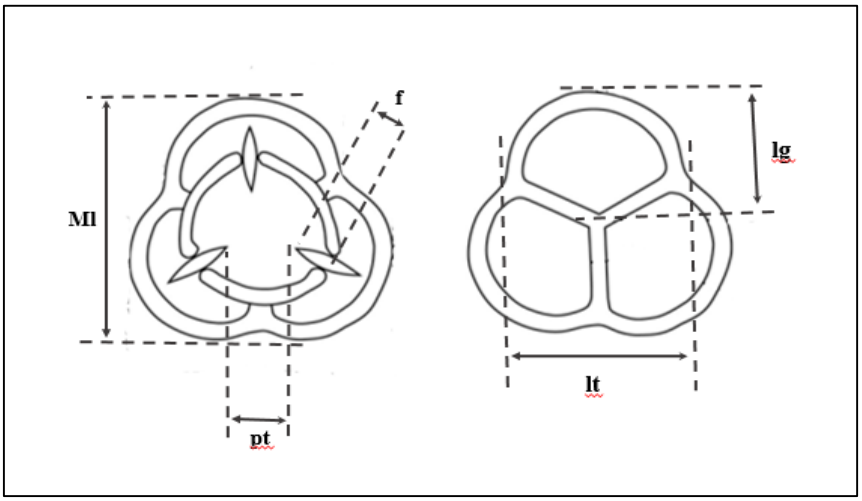


Figure 1. Pollen chart showing measurement values (modified from Sawara 2007)

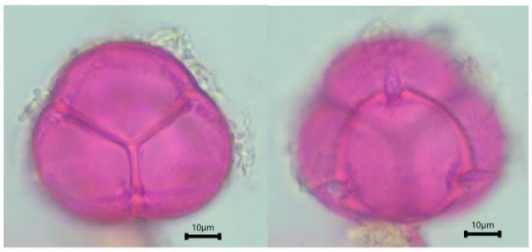
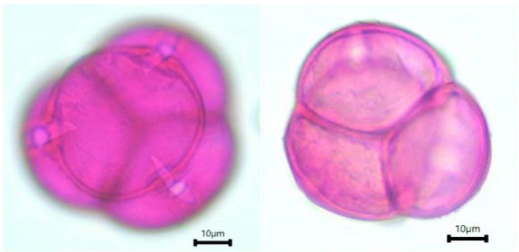
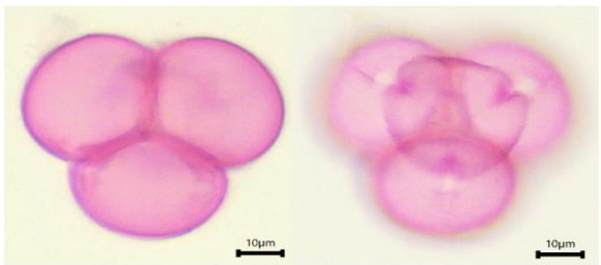
RESULT AND DISCUSSION

Some members of the *Rhododendron* genus are a high source of nectar and pollen, so some species are visited by insects, especially honey bees (Sorkun, 2008; Kurtoğlu et al., 2014). However, the toxic compounds contained in the plant can mix with bee products. It is known that some side effects (poisoning, vomiting, dizziness, hallucinations and even death) occur in the living body due to consuming honey that contains these toxic compounds and is called mad honey (Koca and Koca, 2007). At this point, although the determination of the presence of these compounds in plant nectar by performing chemical analysis in honey seems to be an option, it is not a common and useful method. Unfortunately, research on this is scarce, and detecting these toxic compounds in honey is

difficult. However, melissopalynological studies are a faster and more common method used to diagnose pollen species belonging to this genus and determine their density in honey. In order to understand whether a honey type is monofloral *Rhododendron* honey, the presence of *Rhododendron* spp. in honey should be determined by pollen analysis (Mayda et al., 2018; Özkök et al., 2018; Ecem Bayram et al., 2020). Therefore, morphological examination of *Rhododendron* spp. pollen is important in terms of contributing to systematic studies as well as determining the species containing toxic compounds and the presence of these species in honey. In some parts of eastern Black Sea particularly forest and alpine transition zone (such as Ayder plateau) toxic and nontoxic species of the genus are found together in mixed communities (Figure 2, A ve B). In this study, we investigated 4 different species containing *R. luteum*, *R. ponticum*, *R. ungernii*, R. *caucasicum* (Figure 2, C-F). Pollen photographs and the results of measurements are shown detailed in Table 1.

Figure 2. A-B: mixed *Rhododendron* population in Ayder plateau; C: *R. caucasicum*; D: R. *luteum*; E: *R. ponticum*; F: *R. ungernii.*

Table 1. Pollen photographs and results of measurements

Botanical origin of pollen grains	Microscopic photographs of *Rhodendron* spp. pollen	Measurement and morphological features				
		Ml	Lg	Lt	Pt	f
Rhododendron caucasicum		53.06±3.46 µm	29.9±2.51 µm	41.6±2.96	22.3±1.84 µm	8.5±1.73 µm
Rhododendron luteum		48.26±3.17 µm	26.06±1.28 µm	37.5±2.68 µm	22.23±1.68 µm	7.36±1.44 µm
Rhododendron ponticum		50.69±2.05 µm	26.65±9.35 µm	39.20±1.99 µm	25.87±2.21 µm	7.2±1.09 µm
Rhododendron ungernii		48.33±2.44 µm	26.4±1.41 µm	37.16±2.11 µm	20.3±2.24 µm	7.16±1.00 µm

Pollens belonging to the family can be easily separated from other monad pollens by their tetrahedral tetrad structures. The pollen morphology of *Rhododendron* has been described in many reports (Terzioğlu et al., 2001, Silici et al., 2010; Sarwar and Takahashi, 2013; Ecem Bayram, 2021). Terzioğlu et al., (2001) stated that to make the definitive diagnosis of rhododendrons, wood anatomy and palynological features should be determined as well as their morphological features. Similar to our study, morphological measurement of purple-pink flower *R. ponticum* pollens was also made in their study. The same researchers showed that specimens of *R. ponticum*

with different colors have significant differences in terms of some morphological features (M+, lt, lg, clt and plg). As opposed to the findings of our study, Terzioğlu et al. (2001) found the values of M+, Lg, Lt, Pt, and F to be 56.02, 30.24, 30.24, 28.85, 10.27, respectively, higher than our results.

It was observed that different researchers used different measurement parameters in the measurements of *Rhododendron* pollen. Sarwar and Takahashi (2013) have been reported that 9 measurement parameters are important in the pollen morphology of the *Rhododendron* genus. Those; tetrad diameter (D), polar length (P) and equatorial diameter (d in tetrad or E in monad) of pollen, length (2f in tetrad or L in monad) and width (W) of ectoaperture, length and width of endoaperture, apocolpial and septal exine thickness. Another study that parameters indicated as total length of pollen tetrads (M), width of a pollen in tetrad (lt), height of a pollen in tetrat (lg), semi-length of colpus on a pollen tetrat (f), colpus width (clg), porus length (plg), porus width (plt), one side of triangle polar on a pollen in tetrad (pt), exine thickness (Ex). Sarwar and Sarwar and Takahashi (2013) examined the pollen morphology of 34 *Rhododendron* taxa using a light microscope and scanning electron microscope. Pollen grains are 3-colporate, oblate to suboblate and pollen grains are arranged in tetrahedral tetrads. The apocolpial pollen wall is composed of the exine - well developed tectum, columellae, foot layer and endexine, and the intine. The size of *Rhododendron* pollen tetrads varies widely between 30.9 μm and 67.1 μm (Sarwar and Takahashi 2013). Although the study does not cover the species examined in this study, it is suitable in terms of these features.

As a result, in this study, the pollen morphologies of *Rhododendron* species, a potential source of honey obtained in the Eastern Black Sea Region of Turkey, have been examined and the differences have been revealed. Studies conducted in this way are of particular importance in identifying *Rhododendron* spp. pollen found in different honey types and accurately labelling different honey types. For example, pollen and nectar flow periods of chestnut and rhododendron plants may coincide, and it is seen that the pollen of *Rhododendron* spp. plant gets mixed in the chestnut honey especially in the Black Sea region. In many studies in the literature, it has been reported that honey samples sold as chestnut honey also contain pollens of *Rhododendron* spp. For this reason, honey types that are suspected to contain *Rhododendron* spp. pollen should be put on the market after the necessary palynological analyses are carried out and their concentrations in honey are identified. In addition, this study is an important research that can be a source for the determination of Ericaceae taxa containing grayanatoxin. At this point, the morphological examination of *Rhododendron* pollens carried out in this study will contribute to future research.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ETHICAL STATEMENT

The authors state that no ethical approval was needed.

AUTHORS CONTRIBUTIONS

NM and AÖ performed the analyses; NEB, NM and AÖ contributed to the design and interpretation of the research and to the writing of the paper. All authors read and approved the manuscript.

ACKNOWLEDGEMENT

We thank Golshan Zare for her contributions in the identification of the plants used in the study.

REFERENCES

Anflin, R., Terzioğlu, S. (1994). A new variety of *Rhododendron ponticum* L. *Tur J Agric For,* (18): 137-140.

Avcı, M. (2004). Ormangülleri *(Rhododendron* L.) ve Türkiye'deki doğal yayılışları*, J Geog,* (12): 13-29. ISSN No: 1302-7212.

Baytop T. (1999). Türkiye'de bitkiler ile tedavi, gecmiste ve bugün (Therapy with medicinal plants in Turkey, past and present), 2nd ed. Nobel Tıp Basımevi, Istanbul, Turkey, s. 275.

Çeter, T., Güney, K. (2011). Orman gülü ve deli bal. *U. Bee J.*, 11(4): 124-129.

Chamberlain, D., Hyam, R., Argent, G., Fairweather, G., Walter, K.S. (1996). The genus Rhododendron: its classification and synonymy. Royal Botanic Garden Edinburgh, 181 p.

Cox, P. A., & Cox, K. N. (1997). The encyclopedia of Rhododendron species. Perth: Glendoick, Australia.

Davis, P.H. (1978). Flora of Turkey and the East Aegean Islands. *Rhododendron* L. (ed. P.F. Stevens), Edinburgh, (6): 90-94.

Ecem Bayram, N., Canli, D., Gercek, Y. C., Bayram, S., Celik, S., Güzel, F., Mogil, H., Oz, G. C. (2020). Macronutrient and micronutrient levels and phenolic compound characteristics of monofloral honey samples. *J. Food Nutr. Res.*, 59: 311-322

Ecem Bayram, N. (2021). Vitamin, mineral, polyphenol, amino acid profile of bee pollen from Rhododendron ponticum (source of "mad honey"): nutritional and palynological approach. *J Food Meas Charact*, 15(3): 2659-2666.

Fang, M.Y., Fang, R.C., He, M.Y., Hu, L.C., Yang, H.P. & Chamberlain, D.F. (2005) Rhododendron. In: Zhengyi, W., Raven, P.H., Deyuan, H. (eds.) Flora of China 14. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis. pp. 260–455.

Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç, M.T. (2012) Türkiye bitkileri listesi (damarlı bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını. Flora Dizisi 1, İstanbul.

Koca, I., Koca, A.F. (2007). Poisoning by mad honey: a brief review. *Food Chem Toxicol*, 45(8): 1315-1318, doi: 10.1016/j.fct.2007.04.006.

Kurtoğlu, A.B., Yavuz, R., Evrendilek, G.A. (2014). Characterisation and fate of grayanatoxins in mad honey produced from *Rhododendron ponticum* nectar. *Food Chem*, 161: 47-52, doi: 10.1016/j.foodchem.2014.03.127.

Mayda, N., Özkök, A., Sorkun, K. (2018). Some characteristic properties of chestnut and rhododendron honeys in Turkey. *HJBC*, 46(1): 135-145, Doi: 10.15671/HJBC.2018.223

Milne, R.I., Abbott, R.J., Wolff, K., Chamberlain, D.F. (1999). Hybridization among sympatric species of *Rhododendron* (Ericaceae) in Turkey: morphological and molecular evidence. *Am J Bot*, 86(12): 1776-1785, doi: 10.2307/2656674.

Onat F.Y., Yegen B.C., Lawrence R., Oktay A., Oktay S. (1991), Mad honey poisoning in man and rat. *Rev Environ Health*, 9: 3-9, doi: 10.1515/REVEH.1991.9.1.3.

Özkök, A., Özenirler, Ç., Canli, D., Mayda, N., Sorkun, K. (2018). Monofloral Features of Turkish Honeys According to Mellissopalynologic, Total Phenolic Acid and Total Flavonoid Content. *Gazi Univ J Sci*, 31(3). 713-723.

Sarwar, A.G., Takahashi, H. (2013). "Pollen morphology of Rhododendron L. and related genera and its taxonomic significance." *BJPT,* 20(2): 185-199, doi: 10.3329/bjpt.v20i2.17393

Sawara, A. K. M. (2007). Pollen morphology and its systematic significance in the Ericaceae. PhD Thesis.

Sorkun, K. (2008). Türkiye'nin nektarlı bitkileri, polenleri ve balları. Palme Yayıncılık, p. 248.

Stevens, P. F. 1978. Rhododendron L. In P. Davis, [ed.], Flora of Turkey 6 Edinburgh University Press, Edinburgh, pp 90-94.

Sütlüpınar, N., Mat, A., Satganoglu, Y. (1993). Poisoning by toxic honey in Turkey. *Arch Toxicol*, 67(2): 148-150.

Taşdemir, D., Demirci, B., Demirci, F., Dönmez, A., Başer, K.H.C., Ruedi, P. (2003). Analysis of the volatile components of five Turkish Rhododendron species by headspace solid-phase microextraction and GC-MS (HS-SPME-GC-MS). *Z Naturforsch Sect C J Biosci,* 58(11-12): 797-803, doi: 10.1515/znc-2003-11-1208.

Terzioğlu, S., Merey, N., Anşin, R. (2001). A Study on Turkish *Rhododendron* L. (Ericaceae), *Turk J Agric For,* 25: 311-317.

Wang, Y.G., Li, G.Z., Ou, Z.L. (2006). Pollen morphology of *Rhododendron* and its taxonomic implication. *Guihaia*, *26*: 113-119.

Wodehouse R.P. (1965). Pollen Grain. Hamer Press, New York.

Yıldız, B., Aktoklu, E. (2010). Bitki Sistematiği: İlkin Karasal Bitkilerden Bir Çeneklilere. Palme Yayıncılık, Ankara, pp. 247-249.

Zhang, Y. J., Jin, X. F., Ding, B. Y., Zhu, J. P. (2009). Pollen morphology of *Rhododendron* subgen. Tsutsusi and its systematic implications. *J Syst Evol*, 47(2): 123-138.