

Some vitamin and organic acid contents in the fruits of *Prunus spinosa* L. subsp. *dasyphylla* (Schur) Domin from Europe-in-Turkey

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Abstract

In this study, vitamin A (390 µg), C (7,70 mg), E (2,75 mg), B1 (0,31 mg), B2 (0,80 mg), B6 (0,84 mg), niasin (2,10 mg), folate (24,75 µg) and some organic acid concentrations including malic (158 mg), lactic (126 mg), sitric (44 mg), fumaric (1,64) and quinic acids (non-detectable) in 100 gr dry weight were analysed in the fruits of *Prunus spinosa* subsp. *dasyphyllum*. The valuable contents of the vitamins based on % daily value were obtained with comparison of the recommended intakes for individuals in life stage groups according to dietary reference intakes (FAO/DRIs). Concentration profile of the vitamins compared to the values of the life stage groups were also significantly correlated in general. Investigated traits in the fruits of *Prunus spinosa* subsp. *dasyphyllum* may be significant to explain the chemotaxonomic and evolutionary characteristics as additional parameters and its alternative natural product potential as vegetable genetic resources in the genepool of Anatolia.

Key words: *Prunus spinosa*, fruit, vitamin, organic acid, pharmaceutical products

Introduction

Genus *Prunus* makes up natural hybrids and variable wild populations in Turkey. *Prunus spinosa* as a variable species in its morphological characters covers large distributional areas in Europe, northwestern Africa, Caucasus, Iran and Anatolia Turkey and several infraspecific taxa were described. It is represented by subsp. *dasyphylla* (Schur) Domin in southern and the southeastern part of the range (Browicz 1972; 1996). *Prunus spinosa* subsp. *dasyphylla*, a major constituent of wild plant flora in some regions, have large distributional area in Turkey and wide range of environmental adaptability including resistance to cold, calcareous soils and drought. It is a light requiring and thermophilus species, reaching the elevations of 2200 m. This shrub doesn't occur in the Mediterranean region or in the drier parts of east and south-east Anatolia. It

has certain ecological preferences characterized by a wet climate and grows well in humid places of the western Black Sea, Thrace, Aegean region and central Anatolia (Dönmez and Yıldırım, 2000). Subsp. *spinosa*, a European taxon, differs in having glabrous shoots and leaves (Browicz 1972). In Turkey, a few works on the genus or the species complex were published. In addition to the study of Boissier (1872), an important revision of the genus *Prunus* was prepared by Browicz in the Flora of Turkey (1972). Recently, the taxonomical status of *Prunus* was also discussed in detail using additional parameters (Dönmez and Yıldırım 2000). The range of polymorphism and wide ecological tolerance make complicated the taxonomy of this genus. A large number of intraspecific polymorphisms were detected in the cpDNA (using PCR-RFLP

technics) of the wild shrubs of *P. spinosa* collected from seven deciduous forests across Europea (Mohanty et al. 2000). Native wild forms are also widespread showing wide variability in leaf, petiole and fruit morphology and contain a significant genepool in Anatolia, which is likely to be valuable in breeding improved varieties of plums. The fruits of *P. spinosa* are valuable, both nutritionally and medicinally. It is succulent and far too bitter for human consumption but edible, particularly after it has been frost bitten. It has tonic astringent, diuretic and purgative properties in traditional medicine (Baytop 1999). They also activate metabolism and enhance general resistance. The berries are also used in the making of healthy juices, syrups, jellies, jams. Because of its fruits which contain volatile components rendering it suitable for flavouring the popular alcoholic drinks such as liquers, brandy, sloe gin and wines (Lust 1980; Browicz 1972; 1990; Fernandez-Garcia et al. 1998). The fruit contains large quantities of biologically active substances such as medicinal oil, vitamins, carotene, organic acids etc., making it irreplaceable as a raw material for the pharmaceutical and food industries producing medicinal oil and alcohol-free beverages (Kumarasamy et al. 2004). Turkey is the rich country for the diversity of Drupaceae and the nutritive value of its wild fruits has great importance from the point of being alternative food resources for the populations in rural areas particularly. Ethnobotanical usage of the fruits of *P. spinosa* was also reported from Thrace in Turkey (Kültür 2007). Sugars, organic acids, phenolic compounds, carotenoids and antioxidants like vitamins C and E as natural components of many fruits and vegetables, play important roles in maintaining fruit quality and determining their nutritive and medicinal value. Apart from a few studies on *P. spinosa* from central Anatolia including proximate compositions of the fruits and some technological properties (Çalışır et al. 2005; Marakoğlu et al. 2005), no detailed investigation was reported on the quantitative composition of the pharmaceutical products.

As a variable and common distributed shrub, there may be different genotypes of this taxon in Anatolia exhibiting large diversity of chemotypic characteristics. The raw fruits of *Prunus spinosa* subsp. *dasyphylla* were investigated for some vitamins and organic acid contents in order to determine its product potential as alternative source in the studied population.

MATERIALS AND METHODS

The well-ripened fruit specimens were picked at random from various parts of 20 individual trees of the native population of *Prunus spinosa* L. subsp. *dasyphylla* (Schur Domin) distributed in Lüleburgaz village, the central region of Europea-in-Turkey, in scrubs and forest remnants, field borders, calcerous roadsides, c.100 m. Collected specimens on September were kept in cooled polypropylene bags for transport to the laboratory. They were packed in glass vessels and kept in deep-freeze storage until the analysis was carried out. The seeds were removed from the mesocarps. Vitamin concentrations in 100 gr dry wt. of the fruits of *Prunus spinosa* subsp. *dasyphylla* were compared with the values of estimated average requirements for life stage groups according to dietary reference intakes (FAO/DRIs). Each value determined is the average of duplicate determinations. Statistical analysis of the experimental results at the $p < 0.05$ significance level (SPSS 10.0).

Analytical methods

The levels of β -caroten (AOAC 1995), vitamin E (α -tocopherol) (Manz and Philipp 1998), thiamin (vitamin B1), riboflavin (vitamin B2) (Finglas and Foulks 1984); vitamin B6 (Hoffman et al. 1991), niacin (Windahl et al. 1999) and folic acid (Dionex Vydac Application Note: 9904, 2003) were determined in 100 gr dry weight of the fruits of *Prunus spinosa* subsp. *dasyphylla* by using HPLC according to the standard methods of the instrument. Identification and quantification of vitamins were accomplished by comparing the retention times of the peaks with authentic

standards (Sigma). For HPLC analysis, Shimadzu HPLC (Shimadzu, Classs VP, Kyoto, Japan) was used.

β -caroten: Analysis of β -caroten was carried out on a C₁₈ Vydac 218TP54 column, 250×4.6 mm i.d. (5 μ m particle size), with 100% MeOH as mobile phase at a flow rate of 1 ml/min, column temperature set at 29 °C, and UV/VIS detection was used. The chromatograms were processed at 450 nm wavelengths.

Vitamin E: Vitamin E (α -tocopherol) determination was performed on a 15 cm × 6 mm clc-ods C18 column connected to the fluorescence detector at 295 nm. The mobile phase (100% methanol) was introduced by a solvent delivery pump model LC-10 AD at 1 mL/min.

Vitamin B: For B complex vitamin sample preparation, 30 ml 0.1 M HCl was added and the flask was closed with cotton then with aluminum foil and put in an autoclave. The pH of sample was adjusted to 6.5 and 4.5 with sodium acetate and HCl and the volume was made up with distilled water, filtrated with a normal filter paper. In case of turbidity, the sample was centrifuged to 10 min at 6000 rpm and filtered by using a filter of a 0.45 μ m pore size. A colon oven was used. This had heating and cooling. In HPLC conditions, colon: C18 Omni Sphere 5, 250 x 4.6 mm, λ : 254 nm B6, B2 (riboflavin), folic acid and B1 (thiamin), flowing rate: 1.9 ml/min, injection volume is 20 μ l, mobile phase: 1000 ml phosphate solvent + 360 ml methanol mixture, pressure: 150–160 bar, running time is 22 min.

Folic acid: The detection wavelength was adjusted to 290 nm. The experiment was conducted at 35 °C. The mobile phase for the determination of folic acid was of methanol + 5mM heptane sulphonic acid sodium salt / 0.1%TEA (25:75 V/V). The pH 2.8 was adjusted with orthophosphoric acid. The flow rate was 1 ml/min and the injected volume 10 μ l.

Niacin: The separation was performed at ambient temperature and at a flow rate of 1 ml min⁻¹, using a mobile phase consisting of a 15%

methanol, 85% deionised water mixture containing 0.005M PIC A Reagent. The fluorimetric detector operated at the wavelength of 261 nm. The injection volume was 20 μ l.

Vitamin C: Analysis of vitamin C was performed by the 2,6-dichlorophenolindophenol titration method with UV absorbance detection at 245 nm, in which this dye is reduced by the ascorbic acid, resulting in the disappearance of the color of the dye (AOAC 1995).

Organic acids: Organic acids including malic, lactic, citric, fumaric and quinic acids were determined by HPLC (AOAC 1995). The column (250 × 4.6 mm, ID) packed with 5 μ m Supelcosil LC-8 connected to UV at 210 nm, 2% KH₂PO₄ (pH = 2.3) as mobile phase, at flow rate of 0.5 mL/min, injection volume was 100 μ L. The retention times were compared with that of standard substances (Sigma Chemical Co., St. Louis, MO, USA).

RESULTS

The concentrations of β -carotene (390 μ g), vit.C (7,70 mg), vit.E (2,75 mg), vit.B1 (0,31 mg), vit.B2 (0,80 mg), vit.B6 (0,84 mg), niasin (2,10 mg) and folate (24,75 μ g) were examined in 100 g dry wt. of the fruits of *Prunus spinosa* subsp. *dasyphylla*. Apart from environmental factors and growing conditions, typical vitamin spectrum which may be evaluated in taxonomic delimitation or genotypic characterization were presented in Figure 1. Valuable concentrations of investigated vitamins were also observed compared to dietary reference intakes in different life stage groups (Table 1). B-caroten, vitamin E, B1, B2, B6 and niacin especially exhibit the considerable levels for covering needs of all individuals in the groups documented. The fruits contained higher value than daily requirements of infants for vitamin B1, B2, B6 and Niacin; 1-3 years group for β -carotene, vit.B2, B6 ; and 4-8 years group for vit.B2 and B6. But, vitamin C and folate were quantified at relatively lower levels compared to daily requirements of life stage groups. Correlation analysis was carried out for all vitamin concentrations. Significant correlations were

found between individual vitamin levels (between columns) reflecting the parallel ratios for daily requirements ($p < 0,05$). Concentration profile of the vitamins in *Prunus spinosa* subsp. *dasyphylla* compared to the values of the life stage groups (between lines) were also significantly correlated in the pregnancy ($p < 0,01$) and the other groups in the table ($p < 0,05$), except for the groups of 14-70 y. ($p = 0,057$). Close proportions of Vit.B2:B6, Vit.E:Niacin and Vit.E:B1 compared with the requirements of all life stage groups were detected. Organic acids in the fruits as significant raw materials for the fruit juice industry in the world were ranked as malic acid (158 mg), lactic acid (126 mg), citric acid (44 mg) and fumaric acid 1,64 mg) respectively. No detectable level for quinic acid was examined (Table 2). The differences which could display a variance between specific limits should be considered depending on the genotype and growing conditions of the fruits. Remarkable concentrations of investigated traits in the fruits of this taxon reflect its alternative product potential in nutrition and medicine.

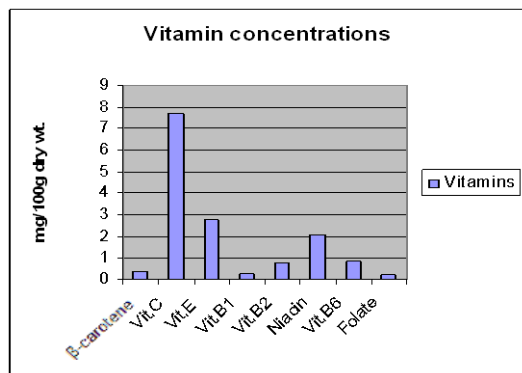


Figure 1. Concentration profile of the vitamins in fruits of *Prunus spinosa* subsp. *dasyphyllum*

DISCUSSION

Five species of *Prunus* including intermediate forms and natural hybrids were reported in the Flora of Turkey. It is probably impossible to distinguish with certainty between truly wild and naturalized populations in Turkey (Browicz

1972). Several infraspecific taxa were also described. Recently, obtained molecular data account for some relations in this genus much more strictly. Microsatellites and cpDNA polymorphisms made possible the analysis of genetic relationships among the *Prunus* accessions and most of the recovered relationships are in agreement with current taxonomic hypotheses and artificial crosses (Bouhadida et al. 2005 2007, Katayama and Uematsu 2005). *P. cerasifera* and *P. spinosa* using RAPD markers were distinct from the European plum group, disproving the hypothesis that *P. x domestica* originated from natural hybrids of *P. cerasifera* and *P. spinosa* (Liu et al. 2006). In the Flora of Turkey, *P. x domestica* which is widely naturalized and possible native was reported as a hybrid between *P. spinosa* and *P. divaricata* that has long been cultivated for its edible fruits. Supportingly, *P. x domestica* was reported to be more closely related to *P. spinosa* than to *P. cerasifera* based on inter-simple sequence repeat markers (Liu et.al, 2007). *P. spinosa* represents one of the possible ancestors of *Prunus domestica* (Watkins 1976; 1981) and are of particular interest for the improvement of rootstocks or varieties through interspecific hybridization. Broad scanning in different populations of *P. spinosa* may provide useful information for the range of genotypic variability. Despite this species exhibits wider variation between local plant groups, many characters were reported to be common in different populations of Turkey and no considerable taxonomical difference from morphological, palinological and anatomical features (Dönmez and Yıldırım 2000). But, in 24 populations of *Prunus spinosa* sampled across Europe, combinations of all the polymorphisms resulted in 33 Chloroplast DNA (cpDNA) haplotypes and two mitochondrial DNA (mtDNA) haplotypes (Mohanty et al. 2003). Furthermore, it was also reported that high polymorphism in the cpDNA of *P. spinosa* has to be considered carefully when planning

Table 1: Vitamin concentrations in 100 gr dry wt. of the fruits of *Prunus spinosa* subsp. *dasyphyllum* compared to adequate intakes*(AIs) and recommended dietary allowances (RDAs) of life stage groups (FAO/DRIs), and their significance levels of correlation

	Vit.A μ g/d	Vit.C (mg/d)	Vit.E (mg/d)	Vit.B1 (mg/d)	Vit.B2 (mg/d)	Niacin (mg/d)	Vit.B6 (mg/d)	Folate (μ g/d)
<i>Prunus spinosa</i> subsp. <i>dasyphyllum</i> (μ g/100g)	390 (β -carotene)	7,70	2,75	0,31	0,80	2,10	0,84	24,75
Infants* (p<0,05)	400-500	40-50	4-5	0,2-0,3	0,3-0,4	2-4	0,1-0,3	65-80
1-3 y. (p<0,05)	300	15	6	0,5	0,5	6	0,5	150
4-8 y. (p<0,05)	400	25	7	0,6	0,6	8	0,6	200
9-13 y. (p<0,05)	600	45	11	0,9	0,9	12	1,0	300
14-70 Female/Male (NS)	400/900	75/90	15	1,1 / 1,2	1,1/1,3	14/16	1,2-1,5/1,3-1,7	400
Pregnancy (p<0,01)	750-770	80-85	15	1,4	1,4	18	1,9	600
Lactation (p<0,05)	1200-1300	115-120	19	1,4	1,6	17	2,0	500

Each value determined is the average of duplicate determinations.

NS: Nonsignificant (p=0,057)

Table 2. Organic acid concentrations in the fruits of *Prunus spinosa* subsp. *dasyphylla* (100 gr dry wt.).

Organic acid	Concentrations (mg/100g)
Malic acid	158,00
Lactic acid	126,00
Citric acid	44,00
Fumaric acid	1,64
Quinic acid	Non-detectable

Each value determined is the average of duplicate determinations

phylogenetic studies involving this species (Mohanty et al. 2000). As a morphologically variable and large distributed species in Anatolia, rich diversity of the wild genotypes should also be considered based on molecular data. On the other hand, very limited number of studies was published for the utility of oil and water soluble vitamin concentrations as chemotaxonomical traits. The tocopherol composition was reported to be used to confirm phylogenetic and taxonomic relations in the plant kingdom (Velasco and Goffman 1999; Goffman et al.1999; Bağcı and Karaağaçlı 2004). The relative content of individual tocopherols is also known to be characteristic of

the seed oil of different cultivated plants. The range of variations in different populations of *Prunus spinosa* is needed to know for utilizing this trait in the characterization of the genotypes. In this study, considerable level of α -tocopherol (2,75 mg/100g) may explain the genotypic features of the investigated population as probably a consistent characteristic, beside to effects of the ripening stage or its growing conditions. Vitamin profiles and their occurrence in the fruits of *Prunus spinosa* may be highly significant and genetically determined indicators of phylogenetic relationships of this genus as additional chemometric data.

Fruits, berries and vegetables contain various phytochemicals with different bioactivities, such as antioxidant activity. The beneficial effects of antioxidants against oxidative stress induced degenerative and age-related diseases have received renewed attention. A functional food provides the body with the required amounts of vitamins, fats, protein, carbohydrates and many other compounds that are needed for its survival. Tocopherols, carotinoids, ascorbic acid, flavonoids, fenolic acids and organic acids are the most important natural antioxidant groups (Meyer et al. 2000). Food analysts have been interested in the amounts of the various chemical components and the changes occurring in the edible parts of fruits, because of their impact on the shelf life, technological and nutritive quality of the food product (Glew et al. 2003). Epidemiological and clinical studies indicate that fruits and vegetable consumption can reduce the risk of several chronic diseases such as cancer, cardiovascular disease, coronary heart disease, and hypertension. Fruits of the plum (*P. x domestica* L.) that is close relative of *P. spinosa* is sweet, juicy and can be consumed freshly in addition to using in different kind of recipes as for valuable nutritional features. There are so many varieties of the plum available. Various types of plums including about 2,000 varieties have different heritages and places of origin. Plums emerged from our food ranking system as a good source of vitamin A (in the form of beta-carotene) and C for providing high ratio of daily value. Comparing with the plums (ESHA foods database- Food Processor for Windows, Version 7.60, by ESHA Research in Salem, Oregon, USA), *Prunus spinosa* in this study exhibits considerably higher levels of the vitamins including vit.C, A, E, thiamin (B1), riboflavin (B2), vit.B6, niacin and folate. As a drought and frost tolerant species, it needs generally no specific cultur conditions apart from some ecological factors and so is a very valuable species that should be evaluated for alternative source potential of the trials examined. From a consideration of

ethnobotanical and taxonomic information, the n-hexane, dichloromethane and methanol extracts of *Prunus padus* and *Prunus spinosa* were the most potent antioxidant extracts (Kumarasamy et al., 2007). It was reported that *Prunus nepalensis* contained 257.1 µg % beta-carotene and 608.9 mg % vitamin C (Agrahar-Murugkar and Subbulakshmi 2005). Compared to above study, considerably higher value for beta-carotene (390 µg/100g dry wt.) and lower levels for vitamin C were quantified for *Prunus spinosa* in this study. Higher vitamin C value of 20 mg/100g from subsp. *dasyphlla* was also reported compared to our measurements resulted in 7,7 mg/100g (Dağlıoğlu and Atansay 1999). This difference may result from the ripening stages or growing conditions of the fruits in addition to genotypic characteristics. It was reported that carotenoid biosynthesis is slow at low temperatures (Britton 1998). Similarly, tomatoes cultivated in the summer presented almost twice the ascorbic acid content as those cultivated during the winter in which the solar incidence is lower (Marchesini and Brugnattelli 1992). Antioxidant vitamins (ascorbic acid, vitamin E, beta -carotene) and phenolic compounds concentration markedly differed among cultivations and it was reported that under the same cultivar and climate conditions, the type of soil management turned out to be of primary importance in influencing the concentration of health-promoting compounds (Lombardi-Boccia et al. 2004). *Prunus spinosa* is widely consumed in the Mediterranean area. As a wild edible gathered for raw consumption or making juices and jams, this species are a good source of nutrients. Considering their low cost and easy availability, it needs to be popularized and recommended for commercial exploitation. However, only a few people like the taste of wild species and many people consider wild food to be old fashioned and prefer to cultivate or buy their food. But, wild foods were traditionally important as a supplement to the diet, particularly during food shortages. *Prunus spinosa* was also used for food in times of scarcity (Luczaj and Szymanski 2007). Comparing to dietary

reference intakes for vitamins, *Prunus spinosa* exhibits valuable contents for daily consumption. Antioxidant vitamins such as vitamin E and beta-carotene resulted at considerable levels. In view of evidence linking folate intake with neural tube defects in the fetus, it is recommended that all women capable of becoming pregnant consume 400 µg from supplements or fortified foods in addition to intake of food folate from a varied diet (FAO/DRIs). The United States introduced mandatory fortification of enriched cereal-grain products with folic acid to reduce the incidence of neural tube defects. As a consequence, substantial amounts of folic acid, the synthetic form of folate, were added to the American diet, and the ability to assess folic acid intake took on greater importance (Póo-Prieto et al., 2006). In this study, considerably high level of folate (24,75 µg/100g dry wt.) in the fruits of *Prunus spinosa* may be evaluated as the alternative source. Intake of folate and vitamin B6 according to recommended dietary allowance were also reported to be important in the primary prevention of coronary heart disease (Rimm et al. 1998). On the other side, the use of therapeutic multivitamins is indicated in cases of deficiency in pathological conditions in which the nutritional requirements are greatly increased. Most of the multivitamin pharmaceutical preparations include water-soluble B-group vitamins. Utility of vitamin B1, B2 and B6 in the antidepressant treatment were also declared (Bell et al. 1992). Vitamin B6 plays an important role in protein, carbohydrate and lipid metabolism. Its major function is the production of serotonin from the amino acid tryptophan in the brain and other neurotransmitters, and so it has a role in the regulation of mental processes. In the body, riboflavin occurs primarily as an integral component of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Obtained levels of vitamin B groups in this study may provide a powerful supplement to the diet. Beneficial effects of niacin therapy were also reported on coronary atherosclerosis (Blankenhorn et al. 1987).

Alternative source potential of *Prunus spinosa* fruits for valuable niacin contents compared to recommended dietary allowances (RDAs) should be considered. In this species, rich contents of vitamin E, C and beta-carotene as free radical scavengers in many kinds of physiological pathways and pathologies may also provide alternative utility. In general, the significant correlation of investigated vitamin contents and their close proportions compared to DRIs values also attract the attention on the healthy and eligible product potential of the fruits. On the other hand, organic acids are involved in various fundamental pathways in plant metabolism and catabolism as intermediate or end products. Some of them among the most abundant, citrate, malate, succinate, fumarate, and acetate in the acetyl coenzyme A form, play a key role in the Krebs cycle which is the central energy-yielding cycle of the cell (Voet and Voet 1998). Some serve as precursor for a variety of products, such as acetate in lipid synthesis or formate in the C1 metabolism. Others, such as malate, are involved in respiration and photosynthesis. Detoxification or incorporation of vital metals bring into play chelating agents such as oxalate or citrate (Jones and Darrah 1994; McCluskey et al. 2004). Organic acids are also responsible for the taste, the flavor, the microbial stability, and the product consistence of plant derived beverages (Arellano et al. 1997; Saavedara and Barbas 2003). Organic acid contents of the fruit of *P. persica* cv. Heitao were ranked as malic acid, citric acid, acetic acid, oxalic acid and succinic acid respectively (Zhong and He 2004). Parallel results were detected in our observations, and malic, lactic, citric and fumaric acids were signified respectively. The quantities of organic acids were reported to vary significantly during the fruit development and malic and citric acids were generally predominant in all development stages in *Diospyros lotus* (Ayaz and Kadioğlu 1998). While the level of malic acid increased continually, the ascorbic acid level decreased dramatically through fruit development in *Mespilus germanica* L. (Glew et al. 2003). The

fruits of *Prunus spinosa* have an alternative potential for the production of organic acids which are among the significant import products of Turkey.

The exploitation and utilization of its natural value may be carried out on the basis of the rich contents. Varietal selection, regional planning and determining the agronomical standards are the key problems. It is also possible to gather the fruits as a raw material in their wild populations which have large distributions in Anatolia. It may be commercialized as juices, jams, fruit ice, gelatins, sweets or liquors. The occurrence of these vitamin components in different genotypes of *Prunus spinosa* as vegetable genetic resources may have practical consequences with respect to plant breeding or genetic engineering and may also attract significant interest with regard to natural product chemistry and plant chemotaxonomy and evolution.

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