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Original article

Evaluating Morphology-based Taxonomic Features for the Identification of Genera within the Anthemidinae (Asteraceae) Subtribe in European Türkiye

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Abstract

The subtribe Anthemedinae, which belongs to the Asteraceae family, encompasses significant taxa with broad distribution. However, the identification, morphological characterization, and diagnosis of these taxa present considerable challenges. In this study, 20 species, subspecies, and varieties from the Anthemis, Cota, Tanacetum, and Tripleurospermum genera, which naturally occur in the European part of Turkey, were investigated. A total of 83 samples were qualitatively and quantitatively measured, focusing on taxonomic features that distinguish the genera. The analysis included an examination of habitus characteristics, life cycle, habit, stem length, branching, and colors. Diagnostic characters of the flowers, such as whether they were discoid or radiate, were determined by measuring the shape, length, and apex structure of the receptacular bracts, as well as the general characteristics of the ligulate flowers. Involucral bracts were analyzed separately as outer, median, and inner, with measurements taken for their shape, size, structure, and apex. Leaves were evaluated separately in terms of shape, fragmentation, and leaflet structures. Achenes were analyzed based on surface structure, size, and the presence or absence of auricle, corona, and gland structures in their apex. The taxonomic features within the determined in this study are useful for developing an identification key for the four genera in the current flora and for distinguishing species in future analyses.

Keywords: Compositae, *Anthemis, Cota, Tanacetum, Tripleurospermum*, Paleae, Phyllaries, Achenes.

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INTRODUCTION

The Anthemideae (Asteraceae) tribe encompasses over 100 genera and around 1800 species (Oberprieler et al., 2007). Species of Anthemideae are predominantly found in Central Asia, the Mediterranean, and South Africa (Oberprieler et al., 2007). Ongoing research on Anthemideae taxa is currently underway in Turkey, leading to the discovery of new species (Özbek et al., 2011; Aytaç et al., 2016; Özbek and Onaylı, 2020; Tekşen et al., 2022). The Anthemidinae subtribe belongs to the Anthemideae tribe and is geographically distributed across Europe, Southwest Asia, North and West Africa, the Canary Islands, and North America (Oberprieler et al., 2022). It comprises species from the genera *Anthemis* L., *Archanthemis* Lo Presti & Oberpr., *Cota* J. Gay, *Nananthea* DC., *Tanacetum* L., *Tripleurospermum* Sch.Bip, and *Xylanthemum* Tzvelev (Oberprieler et al., 2022). This subtribe is comprised of four genera in the region of European Turkey: 1) *Anthemis* L. (*A. arvensis* L., *A. auriculata* Boiss., *A. chia* L., *A. cotula* L., *A. cretica* L. subsp. *pontica* (Willd.) Grierson, *A. cretica* L. subsp. *umbilicata* (Boiss. & A.Huet) Grierson, *A. pectinata* (Bory & Chaub.) Boiss. var. *pectinata*, *A. pseudocotula* Boiss., *A. tomentosa* L. subsp. *tomentosa*), 2) *Cota J.Gay ex Guss.* (*C. altissima* (L.) J. Gay, *C. austriaca* (Jacq.) Sch.Bip., *C. euxina* (Boiss.) U. Özbek & Vural, *C. tinctoria* (L.) J. Gay ex Guss. var. *tinctoria*, *C. tinctoria* (L.) J. Gay ex Guss. var. *pallida* (DC.) Özbek & Vural, *C. tinctoria* (L.) J. Gay ex Guss. var. *discoidea* (All.) Özbek & Vural, *C. triumfettii* (L.) J. Gay ex Guss.), 3) *Tanacetum* L. (*T. balsamitoides* Sch.Bip., *T. corymbosum* (L.) Schultz Bip. subsp. *cinereum* (Gris.) Hayek, *T. parthenium* (L.) Schultz. Bip., *T. vulgare* L.), and 4) *Tripleurospermum* Sch. Bip. (endemic *T. baytopianum* E. Hossain, endemic *T. conoclinium* (Boiss. & Bal.) Hayek, *T. decipiens* (Fisch. & Mey.) Bornm., endemic *T. hygrophilum* (Bornm.) Bornm., *T. inodorum* (L.) Schultz Bip., *T. parviflorum* (Willd.) Pobed., *T. sevanense* (Manden.) Pobed., *T. tenuifolium* (Kit.) Freyn.) (Güner et al., 2012).

The first comprehensive classification of the family into tribes was undertaken by Cassini (1816, 1826). Initially, tribes were divided into two groups based on the presence or absence of paleae, which are receptacular bracts. However, this classification resulted in complexity within subtribes due to other features. Subsequent studies indicated that closely related groups exhibit similar inflorescence epidermis (Bremer, 1987). The initial stage of taxonomic classification for the Anthemidinae subtribe involved morphological characterization (Bremer and Humphries, 1993). However, it was found that morphological findings alone were insufficient for resolving taxonomic issues. Consequently, the use of barcoding-based molecular classification has led to the identification of new monophyletic groups, prompting the recommendation for the incorporation of molecular approaches (Oberprieler et al., 2006; 2007; 2009). The ongoing studies have been essential in addressing the taxonomic ambiguities within the group. The proximity of subtribal taxa poses intricate challenges within the taxonomy field. The intricate interconnections they share often result in complexities in their classification, rendering it challenging to differentiate between taxonomic groups. Additionally, their diverse range of morphological characteristics further contributes to this complexity. Despite the application of various modern methodologies, challenges related to identifying species and subspecies within subtribes endure (Oberprieler, 1998, 2002; Oberprieler et al., 2006; 2007; 2009; Presti et al., 2010; Skilbeck et al., 2019). The variation in habitus and leaf structure among different taxa can often lead to confusion when identifying and distinguishing species. On the other hand, certain species may exhibit high levels of similarity in these characteristics and coexist in the same vegetation, leading to potential confusion in identification (Skilbeck et al., 2019). Morphological characterization of challenging taxonomic groups results in intricate diagnostic criteria. Nevertheless, morphological characterization should not be entirely abandoned in taxonomic classification. Early reports indicated that molecular studies complemented morphology-based classification (Torrell et al., 1999).

Due to increasing human impacts on the environment and other related factors in recent years, both the number of endangered plant species and the loss of biodiversity are on the rise (Kumar et al., 2023; Kolawole and Iyiola, 2023; Pecl et al., 2017; Xu, 2024; Zalles et al., 2021). Many of these plants are considered as weeds in various regions and are subjected to pesticide treatment, but it is recommended to conserve their genetic resources due to their significance in addressing food scarcity and their potential medical benefits. For instance, wild genotypes of Asteraceae taxa have been documented as food sources in Turkey (Şenkardeş et al., 2019). Furthermore, members of the Anthemidinae subtribe, such as *Anthemis arvensis* and *A. cotula*, have been identified for similar purposes (Vučković et al., 2011). Research has indicated that various parts of the *A. chia* plant can serve as antioxidants or anti-diabetic agents in food, cosmetics, and medicine (Sarikurkcu, 2020). Moreover, the use of extracts from the *Cota tinctoria* plant in stomach and liver cancer cell lines may reveal additional medicinal properties of Anthemidinae taxa (Shamloo et al., 2022).

The objective of our research is to conduct a morphological characterization of species naturally occurring in the flora of European Turkey using freshly collected samples. Our aim is to identify new key characteristics that contribute to the complexity of flora records and to conduct preliminary morphological analyses of these traits for advanced molecular studies.

MATERIALS and METHODS

The studied plant samples were gathered from natural populations in European Turkey, with consideration given to their flowering and fruiting periods (Table 1): *Anthemis* (*A. arvensis*, *A. auriculata*, *A. chia*, *A. cretica* subsp. *umbilicata*, *A. cotula*, *A. pectinata* var. *pectinata*, *A. pseudocotula*, *A. tomentosa* var. *tomentosa*), *Cota* (*C. altissima*, *C. austriaca*, *C. euxina*, *C. tinctoria* var. *tinctoria*, *C. tinctoria* var. *pallida*), *Tanacetum* (*T. corymbosum* subsp. *cinereum*, *T. parthenium*), and *Tripleurospermum* (*T. baytopianum*, *T. conoclinium*, *T. decipiens*, *T. hygrophiulm*, *T. tenuifolium*). Sampling was conducted across annual, biannual, and perennial taxa in 5 provinces and 17 districts, spanning altitudes from sea level to 768 m.

Taxonomic identification involved referencing several authoritative sources including the Flora of Turkey and East Aegean Island volume 5 (Davis, 1975), Flora Europaea volume 4 (Tutin et al., 1964), the plant list (Güner et al., 2012), terminology (Güner et al., 2014), and the seed atlas known as Atlas of Seed and Fruits of Central and East-European Flora (Bojnanský and Fargašová, 2007), along with revision studies (Inceer, 2003; Özbek, 2010; Özbek and Vural, 2020). The specimens are deposited in the herbarium or as a PTS (Pelin Turhan Serttas) collection with voucher codes (Table 1). Additionally, monographs on plant nomenclature were reviewed as part of the first author's PhD thesis study (Turhan-Serttaş, 2022). Out of the 346 fresh samples collected, 83 exhibited morphological variations, and detailed qualitative and quantitative measurements were conducted to identify key characteristics. This involved employing the stereomicroscope technique to assess the morphology of the taxa and identify diagnostic features.

Table 1. Collected Anthemidinae taxa examined within the scope of this research.

ID	Taxon	Locality	Voucher
Aarv	***A. arvensis***	Edirne: Between Enez and Yenice Sahil, 40°39'55.59"N 26° 5'29.85"E, oak area, 15 m, 26 v 2019	PTS-19179
	A. arvensis	Edirne: Edirne, between Orhaniye and Elçili, 41°28'54.60"N 26°37'39.56"E, roadside, 31 m, 27 iv 2019	PTS-1929
	A. arvensis	Edirne: Edirne, T.U. Balkan Campus, 70m, 9 v 2020	PTS-204
	A. arvensis	Edirne: Enez, Küçükevren Gülçavuş, 40°37'33.32"N 26°10'57.91"E, pinery, 58 m, 26 v 2019	PTS-19177
	A. arvensis	Edirne: Keşan, Sazlıdere, 40°39'7.71"N 26°41'8.62"E, coastal road, 59 m, 5 v 2019	PTS-19106
	A. arvensis	Edirne: Süloğlu, Süloğlu Dam, 41°47'52.4"N 26°54'51.1"E, dam perimeter, 202 m, 6 iv 2019	PTS-196
	A. arvensis	Tekirdağ: Hayrabolu, Hasköy, 41° 7'25.54"K 26°57'20.27"E, grassland, 182 m, 25 v 2019	PTS-19159
	A. arvensis	Tekirdağ: Hayrabolu, Hasköy, 41° 7'25.54"N 26°57'20.27"E, grassland, 182 m, 25 v 2019	PTS-19157
Aaur	***A. auriculata***	Edirne: Edirne, highway roadside, 60 m, 9 v 2020	PTS-203
	A. auriculata	Edirne: Keşan, Keşan-Gelibolu, 2nd km from Şarköy-Kavak village, roadside, 70 m, 16 v 2020	PTS-207
Achi	***A. chia***	Kırklareli: Kırklareli, 3th km between Üsküp and Çukurpınar, 41°46'53.26"N 27°25'45.19"E, 495 m, 4 v 2019	PTS-1986
Acot	***A. cotula***	Edirne: Lalapaşa, 8th km between Vaysal and Devletli Ağaç, 41°58'12.73"N 26°57'1.88"E, 355 m, 27 vii 2019	PTS-19297
	A. cotula	Edirne: Lalapaşa, Vaysal village, 41°56'31.53"N 26°52'5.45"E, 453 m, 27 vii 2019	PTS-19294
	A. cotula	Edirne: Süloğlu, Süloğlu Dam, 41°47'55.1"N 26°54'52.0"E, dam perimeter, 202 m, 6 iv 2019	PTS-197
	A. cotula	Kırklareli: Demirköy, between Hamamgölü and Sivriler, 41°48'50.85"N 27°55'24.28"E, 144 m, 22 vi 2019	PTS-19254
	A. cotula	Kırklareli: Demirköy, Dayko recreation area, 41°50'21.66"N 27°47'54.37"E, 278 m, 22 vi 2019	PTS-19248

	A. cotula	Kırklareli: Kırklareli, between Demircihalil and Yörükbayırı, 41°49'2.04"N 27°19'19.53"E, pinery, 372 m, 13 vi 2019	PTS-19223
	A. cotula	Kırklareli: Vize, between Yenice and Sergen, 41°42'10.10"N 27°41'31.29"E, 379 m, 15 vii 2019	PTS-19275
	A. cotula	Tekirdağ: Hayrabolu, Avluobası, 41°17'25.46"N 27°14'15.66"E, roadside, 66 m, 25 v 2019	PTS-19137
Acre	***A. cretica* subsp. *umblicata***	Kırklareli: Kırklareli, between Demircihalil and Yörükbayırı, 41°49'2.04"N 27°19'19.53"E, pinery, 372 m, 13 vi 2019	PTS-19229
	A. cretica* subsp. *umblicata	Edirne: Edirne, T.U. Balkan Campus, 41°38'52.74"N 26°37'12.95"E, field, 73 m, 6 xi 2019	PTS-19322
	A. cretica* subsp. *umblicata	Edirne: Edirne, T.U. Balkan Campus, 41°38'52.74"N 26°37'12.95"E, field, 73 m, 9 xi 2019	PTS-19323
Apec	***A. pectinata* var. *pectinata***	Edirne: Between Enez, Küçükevren Gülçavuş, 40°37'33.32"N 26°10'57.91"E, pinery, 58 m, 26 v 2019	PTS-19176
	A. pectinata* var. *pectinata	Edirne: Enez, Yenice Dam, 40°41'56.35"N 26° 9'15.91"E, dam perimeter, 91 m, 26 v 2019	PTS-19180
Apse	***A. pseudocotula***	Çanakkale: Gökçeada, Şirinköy, 40º 07' 52"N 25º 44' 37"E, field, 60 m, 7 vi 2019	PTS-19196
	A. pseudocotula	Kırklareli: Vize, 7th km between Yenice and Sergen, 41°42'10.10"N 27°41'31.29"E, 379 m, 15 vii 2019	PTS-19275.1
Atom	***A. tomentosa***	Çanakkale: Gökçeada, Uğurlu, Mavisu Resort hotel, 40° 7'6.21"N 25°41'59.03"E, beach, 15 m, 7 vi 2019	PTS-19199
Calt	***C. altissima***	Çanakkale: Gökçeada, Şirinköy, 40º 07' 52"N 25º 44' 37"E, field, 60 m, 7 vi 2019	PTS-19197
	C. altissima	Çanakkale: Gökçeada, Şirinköy, 40º 07' 52"N 25º 44' 37"E, field, 60 m, 7 vi 2019	PTS-19198
	C. altissima	Istanbul: Beşiktaş, Yıldız Grove, 41° 2'48.57"N 29° 0'56.51"E, grove, 45 m, 2 vi 2019	PTS-19182
	C. altissima	Kırklareli: Babaeski, between Babaeski and Karamesutlu, 7th km, 41°28'4.62"N 27° 5'50.02"E, wetland, 70 m, 28 ix 2019	PTS-19318
	C. altissima	Kırklareli: Kırklareli, Karlıktepe, 41°53'3.51"N 27°28'19.38"E, hill, 553 m, 13 vi 2019	PTS-19230
	C. altissima	Tekirdağ: Hayrabolu, Avluobası Village, 41°17'25.46"N 27°14'15.66"E, roadside, 66 m, 25 v 2019	PTS-19138
	C. altissima	Tekirdağ: Hayrabolu, between Tatarlı Village and Canhıdır, 41° 8'35.34"N 27° 4'46.82"E, black bush, 118 m, 25 v 2019	PTS-19145
	C. altissima	Tekirdağ: Hayrabolu, between Umurbey and Kutlugün, 41° 4'28.84"N 27° 1'42.19"E, 116 m, 25 v 2019	PTS-19151
	C. altissima	Tekirdağ: Hayrabolu, Küçükkarakarlı, 41°19'5.43"N 27°11'59.40"E, roadside, 45 m, 25 v 2019	PTS-19135
	C. altissima	Tekirdağ: Hayrabolu, Umurbey dam, 41° 3'52.51"N 27° 2'24.96"E, dam perimeter, 103 m, 25 v 2019	PTS-19148
Caus	***C. austriaca***	Edirne: Edirne, Hasanağa-Musabeyli road, 41°40'49.5"N 26°34'51.9"E, roadside, 129 m, 6 iv 2019	PTS-1911
	C. austriaca	Edirne: Edirne, road side, 2 vi 2020	PTS-211
	C. austriaca	Edirne: Edirne, road side, 9 v 2020	PTS-201
	C. austriaca	Edirne: Edirne, T.U. Balkan Campus, 41°38'52.74"N 26°37'12.95"E, 73 m, 19 v 2019	PTS-19121

	C. austriaca	Edirne: Edirne, T.U. Balkan Campus, 41°38'52.74"N 26°37'12.95"E, field, 73 m, 9 xi 2019	PTS-19327
	C. austriaca	Edirne: Edirne, T.U. Balkan Campus, 41°38'52.74"N 26°37'12.95"E, field, 73 m, 6 xi 2019	PTS-19321
	C. austriaca	Edirne: Edirne, T.U. Balkan Campus, 41°38'52.74"N 26°37'12.95"E, 73 m, 19 v 2019	PTS-19120
	C. austriaca	Kırklareli: Kırklareli, between Üsküpdere and Yündalan, Üsküp road, 41°42'55.16"N 27°22'16.37"E, 274 m, 4 v 2019	PTS-1992
	C. austriaca	Kırklareli: Kofçaz, 10th km between Topçular and Ahmetler, 42° 1'26.66"N 27°12'13.99"E, 610 m, 27 vii 2019	PTS-19306
	C. austriaca	Kırklareli: Vize, 7th km between Yenice and Sergen, 41°42'10.10"N 27°41'31.29"E, 379 m, 15 vii 2019	PTS-19276
Ceux	***C. euxina***	Tekirdağ: Saray, Kasatura, 12 vi 2020	PTS-215
	C. euxina	Tekirdağ: Saray, Kasatura bay, 41°35'5.44"K 28° 8'33.92"D, dunes, 15 vii 2019	PTS-19285
Ctin	***C. tinctoria* var. *tinctoria***	Edirne: Edirne, T.U. Balkan Campus, 41°38'29.61"N 26°37'10.89"E, 56 m, 21 v 2019	PTS-19123
	C. tinctoria* var. *tinctoria	Edirne: Enez, Gala gölü, Hisarlıdağ Tepesi, 40°45'0.89"K 26°11'1.19"D, tepe, 61 m, 26 v 2019	PTS-19167
	C. tinctoria* var. *tinctoria	Kırklareli: Demirköy, Mahyadağ, 41°45'28.90"N 27°39'43.91"E, 768 m, 22 vi 2019	PTS-19235
	C. tinctoria* var. *tinctoria	Kırklareli: Kırklareli, t6 road, roadside, 12 vi 2020	PTS-213
	C. tinctoria* var. *tinctoria	Kırklareli: Vize, 9. km between Sergen and Kızılağaç, 41°41'55.18"N 27°46'24.00"E, 404 m, 15 vii 2019	PTS-19272
	C. tinctoria* var. *tinctoria	Tekirdağ: Hayrabolu, between Hasköy and Subaşı, 41° 7'25.54"N 26°57'20.27"E, pasture, 182 m, 25 v 2019	PTS-19156
	C. tinctoria* var. *tinctoria	Tekirdağ: Hayrabolu, Kutlugün pond, 41° 5'24.85"N 26°58'0.71"E, oak grove, 125 m, 25 v 2019	PTS-19154
Cpal	***C. tinctoria* var. *pallida***	Kırklareli: Demirköy, between Mahyadağı and Demirköy, 41°46'32.07"N 27°41'58.02"E, 602 m, 22 vi 19	PTS-19246
	C. tinctoria* var. *pallida	Kırklareli: Kırklareli, between Çağlayık and Dereköy, 42° 0'13.61"N 27°22'51.54"E, 553 m, 27 vii 2019	PTS-19310
	C. tinctoria* var. *pallida	Kırklareli: Kırklareli, between Çağlayık and Dereköy, 42° 1'8.61"N 27°21'42.89"E, 413 m, 27 vii 2019	PTS-19307
	C. tinctoria* var. *pallida	Kırklareli: Kırklareli, between Demircihalil and Yörükbayırı, 41°49'2.04"N 27°19'19.53"E, pinery, 372 m, 13 vi 19	PTS-19224
	C. tinctoria* var. *pallida	Kırklareli: Kırklareli, Karlıktepe, 41°53'33.02"N 27°28'56.95"E, hill, 624 m, 13 vi 19	PTS-19233
Tcor	***T. corymbosum* subsp. *cinereum***	Kırklareli: Kırklareli, between Demircihalil and Yörükbayırı 41°49'2.04"N 27°19'19.53"E, pinery, 372 m, 13 vi 2019	PTS-19226
	T. corymbosum* subsp. *cinereum	Kırklareli: Demirköy, Mahyadağ, 41°45'28.90"N 27°39'43.91"E, 768 m, 22 vi 2019	PTS-19238
	T. corymbosum* subsp. *cinereum	Kırklareli: Kırklareli, between Kapaklı and Koruköy, 41°52'52.64"N 27°20'5.00"E, quarries, 538 m, 13 vi 2019	PTS-19216
	T. corymbosum* subsp. *cinereum	Kırklareli: Vize, Akıncılar Village, 41°28'18.58"N 27°40'7.76"E, oak, 163 m, 8 vi 2019	PTS-19208
	T. corymbosum* subsp. *cinereum	Tekirdağ: Saray, Bahçeköy road, 41°33'21.30"N 28°03'35.99"E, roadside, 157 m, 12 vi 2020	PTS-212

	T. corymbosum* subsp. *cinereum	Tekirdağ: Saray, Kasatura exit, 41°35'31.38"N 28°08'25.11"E, roadside, 24 m, 12 vi 2020	PTS-216
Tpar	***T. parthenium***	Kırklareli: Demirköy, Mahyadağ, 41°45'28.90"N 27°39'43.91"E, 768 m, 22 vi 2019	PTS-19237
	T. parthenium	Kırklareli: Demirköy, between Mahyadağı and Demirköy, 41°46'32.07"N 27°41'58.02"E, 602 m, 22 vi 2019	PTS-19241
Tbay	***T. baytopianum***	Edirne: İpsala, Turpçular, 40°56'44.17"N 26°26'42.31"E, pasture, 61 m, 28 iv 2019	PTS-1971
	T. baytopianum	Edirne: İpsala, Turpçular, 40°56'44.17"N 26°26'42.31"E, pasture, 61 m, 28 iv 2019	PTS-1968
	T. baytopianum	Kırklareli: Lüleburgaz, between Oklalı and Küçükkarakarlı, 41°19'49.78"N 27°12'39.77"E, pasture, 57 m, 25 v 2019	PTS-19132
	T. baytopianum	Edirne: Keşan, between Yerlisu and Keşan, 40°45'4.66"N 26°42'51.13"E, red pine, roadside, 83 m, 5 v 2019	PTS-1995
	T. baytopianum	Edirne: Keşan, between Yerlisu and Keşan, 40°45'4.66"N 26°42'51.13"E, roadside, 83m, 5 v 2019	PTS-1994
	T. baytopianum	Edirne: Edirne, T.U. Balkan Campus, 41°38'46.00"N 26°37'12.74"E, arboretum, 62 m, 4 iv 2019	PTS-195
Tcon	***T. conoclinium***	Kırklareli: Demirköy, Dayko, 41°50'21.66"N 27°47'54.37"E, 278 m, 22 vi 2019	PTS-19247
Tdec	***T. decipiens***	Edirne: Uzunköprü, Historical bridge, 41°16'12.15"N 26°40'59.09"E, cut stones, 18 m, 24 vi 2019	PTS-19257
Thyg	***T. hygrophilum***	Kırklareli: Lüleburgaz, Oklalıköyü, 41°20'9.80"N 27°13'14.91"E, pasture, 60 m, 25 v 2019	PTS-19130
Tten	***T. tenuifolium***	Kırklareli: Kırklareli, between Armağan and Dereköy, 41°53'29.22"N 27°24'5.35"E, 495 m, 13 vi 2019	PTS-19221
	T. tenuifolium	Kırklareli: Kırklareli, between Kapaklı and Koruköy, 41°52'52.64"N 27°20'5.00"E, quarries, 538 m, 13 vi 2019	PTS-19212
	T. tenuifolium	Kırklareli: Kofçaz, 10th km between Topçular and Ahmetler, 42° 1'26.66"N 27°12'13.99"E, 610 m, 27 vii 2019	PTS-19305
	T. tenuifolium	Kırklareli: Kofçaz, between Malkoçlar and Beyciler, 42° 2'21.83"N 27° 3'43.87"E, 352 m, 27 vii 2019	PTS-19299
	T. tenuifolium	Kırklareli: Kofçaz, Topçular - Ahmetler road, 42° 2'37.63"N 27° 8'21.18"E, 565 m, 27 vii 2019	PTS-19304

RESULTS and DISCUSSION

In this study, we conducted both qualitative and quantitative measurements, along with a thorough morphological analysis, of 83 samples representing 20 different taxa from the 4 genera. These taxa are part of the Anthemidinae subtribe and are naturally growing in the European region of Turkey.

1. Paleae present in the receptacle

2. Achene pressed; flattened; marginal coronate; non-obvious rib **2. Cota**

2+ Achene cylindrical; terete or circular; certain coronate or airucle present or absent; obvious rib, obsolete or tuberculate........**1. Anthemis**

1+ Paleae absent in the receptacle

3. Leaves pinnatisect, fimbriate segments, linear-laciniate; achene with 2 glands or eglandular in mature; 3 dorsal ribs...... **4. Tripleurospermum**

3+ Leaves strongly pinnatisect, obovoid-lanceolate segments; achene without gland; both surfaces with 5-10 rib... **3. Tanacetum**

The identification key for Anthemidinae genera in the flora of the European Turkey was updated based on Davis (1965).

Upon analyzing the characterization data for the overall appearance of the plants, it was observed that the plants encompassed annual, biannual, and perennial categories in terms of their life spans (Table 2). Specifically within the *Anthemis* genus, it was noted that the perennial plants *A. pectinata* var. *pectinata* and *A. cretica* subsp. *umblicata* exhibit an erect habitus, while the others display a decumbent or suberect posture. The range of surface pubescence varied from tomentose and pubescent to glabrescent. Additionally, while the *Cota* genus can be divided into perennial and annual categories, the distinction is based on flower, achene, and leaf morphologies. Although the *Tanacetum* genera consist of perennial species, no annual taxa were found within the members of the *Tripleurospermum* genus. In conclusion, it was determined that habitus characteristics alone are insufficient to fully differentiate the various species within themselves.

Table 2. Comparison of habitus characteristics of Anthemidinae taxa in natural habitat.

Taxon*	Life cycle	Habit	Length (cm)	Stem	Indumentum	Color
Aarv	Annual	Erect, decumbent	24-32.5	Sparsely	Subpubescent	Grey-green to green
Aaur	Annual	Erect, suberect	(19-)20-(39)50	Sparsely	Pubescent	Grey-green or green
Achi	Annual	Erect, suberect	18.5-35	Branches from the base	Pubescent	Green to light green or almost light green
Acot	Annual	Erect to suberect	20-45	Brached	Glabrescent or subpubescent	Green or light green
Acre	Perennial	Erect	21-41	Branches from the base	Pubescent	Grey-green
Apec	Perennial	Erect	15-30	Caespitose	Pubescent of base, glabrescent of top	Green
Apse	Annual	Erect to decumbent	10-24.5	Brached	Subpubescent	Green
Atom	Annual	Adpressed	10-25	Sparsely to simple	Lanate to tomentosa	Grey-green to whitish green
Calt	Annual	Erect to decumbent	30-85	Brached	Subglabrescent	Brownish green to green
Caus	Annual	Erect to decumbent	23-87	Brached	Subpubescent to pubescent	Green or sometimes grey green
Ceux	Perennial	Erect or decumbent	28.7-35	Branches from the base	Long pubescent	Grey-green to green
Ctin	Perennial	Erect	40-54(-60)	Branches from the base	Pubescent or subpubescent	Grey-green to green
Cpal	Perennial	Erect	28.5-53.5	Branches from the base	Pubescent or subpubescent	Grey-green to green
Tcor	Perennial	Erect	37-75	Densely brached	Glabrescent to subglabrescent	Green or grey-green to dark green
Tpar	Perennial	Erect	45-57	Densely brached	Glabrescent to subglabrescent	Green
Tbay	Biannual, perennial	Erect	17-45	Simple brached	Subglabrescent to glabrescent	Brownish green to green
Tcon	Biannual, perennial	Erect	55	Simple brached	Subglabrescent to glabrescent	Green
Tdec	Biannual	Erect	10-90(-100)	Simple brached	Subglabrescent to glabrescent	Green
Thyg	Perennial	Erect	25-52	Simple brached	Subglabrescent	Green or brownish green
Tten	Perennial	Erect	55-130	Densely brached	Subglabrescent to glabrescent	Green

*For taxon list see Table 1.

The discoid variations of *A. pectinata* and *T. decipiens* can be differentiated from other taxa by observing the condition of the ligula (Figure 1, Table 3). Even with limited information about other attributes, specific characteristics alone may suffice to distinguish between taxa. In particular, *A. pectinata*, a perennial component of the *Anthemis* genus, exhibits a distinguishing feature from the *T. decipiens* taxon with the inclusion of paleae (receptacular bracts), thereby distinguishing it from other species within the group (Figure 1E and 1O). *Cota* genus is recognized for its large receptacles when mature capitula are present (Figure 1F-J). Variations in leaf morphology can be used to distinguish species within this taxonomic group, particularly those characterized by a yellow ligula. Distinctive traits were identified in the annual taxa of *Anthemis* genus. Specifically, *A. cotula* species displays a smaller structure in its habitus due to its differently positioned paleae structure. In contrast, a corymbose structure is evident in members of *Tanacetum* genus.

When examining paleae, it is possible to distinguish between *Anthemis* and *Cota*, *Tanacetum* and *Tripleurospermum* based on their presence or absence, which is an important factor in the diagnostic key. Within the Anthemidinae subtribe, the paleae of *A. cotula* and *A. pseudocotula* species are linear, with a width and length ratio of 1/15, and are located in the middle part of the receptacle, lacking any bract structure on the margins. The paleae morphology of annuals in the genus *Cota* allows for the evaluation of the paleae tip as ½ and ¼, respectively, which aids in distinguishing *C. altissima* from *C. austriaca*.

Figure 1. Capitula form of some Anthemidinae taxa. A- A. arvensis; B- A. auriculata; C- A. chia; D- A. cotula; E- A. pectinata var. pectinata; F- C. altissima; G- C. austriaca; H- C. euxina; I- C. tinctoria var. tinctoria; J- C. tinctoria var. pallida; K- T. corymbosum subsp. cinereum; L- T. parthenium; M- T. baytopianum; N- T. hygrophilum; O- T. decipiens; P- T. tenuifolium.

Table 3. Diagnostic morphological features of flowers in Anthemidiniae taxa. Capitula: Type / branching / number / mature receptacle shape / radius measurement size with ligulate flower (cm). Involucre: radius measurement size (cm). Paleae: absent or present (shape / size (mm) / apex). Ligules: Flower (number / color / size (mm)).

Taxon*	Capitula	Involucre	Paleae	Ligules
Aarv	Radiate / scattered / single / acute dome-shaped / 1.5-3	1-1.5	Present: oblanceolate to (or) spatulate / 2.5 to 5 / acuminate, taper-pointed (0.85 mm)	12-21 / white / 7-12 x 2-4.6
Aaur	Radiate / scattered / single / subconical / 3-3.5	1-1.5	Present: oblanceolate to oblong / 4.3 / moucronate	18-20 / white / 5-12.5
Achi	Radiate / apexial / single / sphaerical / 2.5	1.2	Present: oblong-lanceolate / 3.4 / acute or moucronate	15-20 / white / 7.5-12.5
Acot	Radiate / separated-scattered / furcated / circular to conical / 1-2	0.7-1	Present: linear-subulate / 2-3 / acute	11-15 / white / 4-8 x 3-5.75
Acre	Radiate / simple / single / hemisphaerical / 1.3	0.65	Present: subulate / 4 / acute-acuminate	12 / white / 7 x 3
Apec	Discoid / apaxial / single / circular / 0.75-1	0.75-1	Present: thin oblanceolate / 2.5 / spine acuminate	Non ligulate
Apse	Radiate / simple / scattered / conical / 2	0.5-0.75	Present: thin oblanceolate / found just in the middle of the receptacle, not at the margins	12 / white / 7 x 3
Atom	Radiate / apexial / sparsely conical / 1.1	1	Present: oblong or ovoid / 4.5 / moucronate scarious and dark color on the middle	10 / white / 4.5 x 3.5
Calt	Radiate / apexial or marginal / campanulate / 3-4.75	1.5-2	Present: linear-oblong / 4.5-7 / apex ½ acuminate (2-4.5 mm)	19-22 / white / 8-22.3 x 3.8-5.5
Caus	Radiate / terminal / scattered or rarely single, sparsely subcorymbose / sphaerical / 2.75-3.5	1.25-1.3	Present: linear-oblanceolate or oblong / 4-6 / apex ¼ acuminate (0.75-1.05 mm)	20-24 / white / 6.5-12 x 2.85-5
Ceux	Radiate / terminal / subcircular / 3	1.3-1.75	Present: oblong / 4-5 / acuminate (1-2 mm)	23 / yellow / 6-6.5 x 2.5-4.25
Ctin	Radiate / terminal / single / flat or subcircular / 2-3.5	1.3 to 2	Present: linear-oblong or oblanceolate / 3.25-5 / acuminate	19-32 / yellow / 6.35-17 x 3.2-4
Cpal	Radiate / terminal / single / flat to circular / 3	1.2-1.75	Present: oblong / 3.7-4.5 / acuminate	23-33 / white, whitish or light yellow / 6-10 x 2.5-4
Tcor	Radiate / terminal / densely corymbose / flat / 1-1.5	0.8-0.85	Absent	14 / white / 6-8 x 3-3.75
Tpar	Radiate / terminal / sparsely corymbose / flat / 1.5	0.7	Absent	11-15 / white / 6-7 x 3
Tbay	Radiate / terminal / single / sparsely subconical / 1.5-2 (glandular)	0.95-1.25	Absent	17 / white / 6-8.5 x 2.75-3

Tcon	Radiate / terminal / sparsely corymbose / flat / (glandular)	0.9-1.5	Absent	18 / white / 9-13
Tdec	Discoid / scattered / circular / 1 (glandular)	1	Absent	Non ligulate
Thyg	Radiate / terminal / single / hemisphaerical / (glandular)	1-1.8	Absent	15-18 / white
Tten	Radiate / terminal / sparsely corymbose / 2-2.7 (glandular)	1.3-2.5	Absent	22-37 / white / 7 x 0.37

*For taxon list see Table 1.

In the macromorphological analysis of the involucral bracts of four genera within the Anthemidinae subtribe in European Turkey, we considered quantitative measurements of the outer, median, and inner phyllaries (Table 4). We have identified key characteristics in the phyllary structures of certain taxa. Notably, the ciliate apex on the inner phyllaries was found to be significant, particularly in differentiating between *C. altissima* and *C. austriaca*, which are annual species in the genus *Cota*. *C. austriaca* has an acute-subobtuse apex with a 0.5 mm ciliate structure, while *C. altissima* has an obtuse hyaline apex and scarious margin. Furthermore, while the ciliate structure was observed in the genus *Cota*, the ciliolate apex was seen in the subspecies *A. cretica* subsp *umblicata*, a perennial taxon in the genus *Anthemis*. However, this ciliolate feature appears weaker compared to the ciliate structure in the genus *Cota*.

Table 4. Diagnostic morphological features of involucre in Anthemidiniae taxa. Shape, size (mm), structure and apex.

Taxon*	Outer Phyllaries	Median Phyllaries	Inner Phyllaries
Aarv	Lanceolate to ovoid, 3.15-6 x 1-2, submoucronate	Oblong to lanceolate, 4-6.3 x 1.5-2.1, acute-acuminate	Oblong-spatulate, 6-7 x 2-3, hyaline
Aaur	Ovoid-lanceolate, 3.5 x 1.5, acute hyaline	Oblong-lanceolate, 4 x 1.5, subacute	Obovoid or oblanceolate, 6 x 3, glabrescent
Achi	Ovoid, 2.5 x 1.5, acute hyaline	Oblong, 4 x 2, scarious	Oblong, 4.5 x 2, acute-subacute hyaline
Acot	Ovoid-oblong, 1.65-3 x 0.85-1.5, subacute glabrescent and scarious	Lanceolate or oblong-lanceolate, 2.75-3.75 x 0.95-1.35, glabrescent submoucronate	Oblong or oblanceolate, 3.3-4 x 1.25-1.8, subglabrescent and hyaline scarious
Acre	Ovoid, 3 x 1.2, acute pubescent	Oblanceolate, 3.2 x 1.3, subpubescent and acute	Linear-oblong, 4.1 x 1.1, acuminate and ciliolate
Apec	Lanceolate-ovoid, 3.75-4 x 1.25-1.5, glabrescent and acute hyaline-scarious	Oblanceolate, 4.5-5.5 x 2, glabrescent acute-subacute and scarious	Oblong-oblanceolate, 6 x 2.75, scarious
Apse	Lanceolate or oblanceolate, submoucronate or acute, scarious	Ovoid-oblong, pubescent, acute or subacute, scarious	Ovoid-oblong, pubescent, acute or subacute, scarious

Atom	Lanceolate-ovoid, 3.5 x 1.3, pubescent-subpubescent and acute scarious	Oblanceolate-subulate, 4 x 1.5, subpubescent and acute scarious	Oblong-oblanceolate, 5 x 1.7, subpubescent-glabrescent, acute-obtuse and scarious
Calt	Lanceolate-ovoid or ovoid, 2.15-5 x 1-2.1, sparsely subpubescent and acute-subobtuse scarious, small ciliate	Lanceolate-ovoid or oblong-lanceolate-ovoid to oblong, 4.25-6.5 x 1.3-2.5, pubescent to glabrescent, subacute to obtuse, non ciliate scarious	Linear-oblong or oblong-lanceolate-ovoid, 5.5-8 x 1.85-3.15, sparsely pubescent to glabrescent, subacute to obtuse hyaline and scarious
Caus	Laceolate-ovoid, 3-4.5 x 1-1.75, pubescent to subpubescent and acute with ciliate, scarious	Lanceolata or oblong-lanceolate, 4-5.5 x 1.1-1.8, sparsely pubescent and acute-subacute with ciliate and scarious	Lanceolate, oblanceolate or oblong-elliptic, 4.15-6.25 x 1.13-1.7, subpubescent-glabrescent, acute-subobtuse with 0.5 mm ciliate apex
Ceux	Lanceolate-ovoid, 3.5-3.75 x 1-1.25, pubescent with acute and rarely ciliate	Lanceolate or oblong-lanceolate, 4.6 x 1.4, pubescent with acute-subobtuse and ciliate	Oblong, 5.5 x 1.4, pubescent to subpubescent, subacute with ciliate
Ctin	Ovoid or lanceolate-ovoid, 3-4.5 x 1-1.5, pubescent with acute to subacute or subobtuse and brownish ciliate	Lanceolate-ovoid or oblanceolate, 4-6.25 x 1.2-1.75, subpubescent with acute to subobtuse with ciliate scarious	Lanceolate or oblong, 5-7 x 1.2-2, subpubescent with acute to obtuse and brownish ciliate
Cpal	Ovoid or lanceolate-ovoid, 3.5-5 x 1.5-2, pubescent with acute to subacute-obtuse and ciliate	Lanceolate or oblong (ovoid), 4.5-6 x 1.5-2, subpubescent with subacute and ciliate	Oblong or oblong-linear, 5-6.5 x 1.5-2, sparsely subpubescent with subobtuse and ciliate
Tcor	Ovoid or lanceolate, 2.5-3.1 x 1-1.35, pubescent or subpubescent with acute, subacute or obtuse and scarious	Oblongs (ovoid), 3.5-4 x 1.2-1.7, subpubescent with acute to obtuse and scarious	Oblong-spatulate, 4-6 x 1.35-2, subpubescent, subacute to obtuse and scarious
Tpar	Lanceolate or ovoid-lanceolate, 2-2.3 x 0.75-0.85, subglabrescent with carinate, subacute-obtuse and scarious	Oblong, 2.6-2.75 x 0.8-1.1, glabrescent, obtuse	Oblong-lanceolate or linear, 3 x 0.65-1, subpubescent, subobtuse, scarious
Tbay	Oblong, lanceolate-ovoid, 3-3.5 x 1-1.5, subglabrescent, subacute to subobtuse	Oblong to oblanceolate, 4 x 1.1-1.75, subglabrescent, acute to subobtuse	Oblong-oblanceolate ot spatulate, 3.3-4.3 x 1-2, glabrescent, obtuse
Tcon	Ovoid-oblong, obtuse or subotuse, pubescent to glabrescent, brownish hyaline	Ovoid-oblong, obtuse or subotuse, pubescent to glabrescent, brownish hyaline	Ovoid-oblong, obtuse or subotuse, pubescent to glabrescent, brownish hyaline
Tdec	Ovoid or lanceolate-ovoid, 2.75 x 2, glabrescent, subacute to obtuse	Lanceolate-ovoid, 3.25 x 1, glabrescent, subacute-obtuse and scarious	Oblong, 4 x1, glabrescent, obtuse
Thyg	Oblong, oblong-lanceolate, obtuse or subobtuse, brownish scarious, glabrescent	Oblong, oblong-lanceolate, obtuse or subobtuse, brownish scarious, glabrescent	Oblong, oblong-lanceolate, obtuse or subobtuse, brownish scarious, glabrescent
Tten	Lanceolate-ovoid or rarely ovoid, 3-4.75 x 1.45-2, glabrescent, acute, scarious margin	Oblong-lanceolate, lanceolate or lanceolate-ovoid, 4-4.3 x 1.25-1.75, acute or acute-obtuse, scarious	Oblong or oblong-oblanceolate, 4.5-5 x 1.2-2, acute or subobtuse

*For taxon list see Table 1.

The leaf shapes of Anthemidinae species vary significantly; even among those that are not closely related (Figure 2, Table 5). We analyzed the fragmentation numbers of middle leaves from various specimens as morphological diagnostic criteria. We found that the leaves morphology of the *A. pectinata* species is particularly distinctive (Figure 2F). Additionally, we observed the densely tomentose in the indumentum of *A. tomentosa* leaves, while *A. pectinata* species exhibited a glabrescent or subglabrescent surface morphology. Overall, the leaf morphology within *Anthemis* taxa was generally characterized as oblanceolate or oblong-lanceolate (Figure 2A-F). Leaves morphology of the *Cota* genus exhibited a general similarity. Both examined varieties of *C. tinctoria* displayed very similar morphologies (Figure 2I). The foliage of *C. austriaca* exhibited certain resemblances to specific genotypes of *C. tinctoria*. However, *C. tinctoria* is distinguished by its relatively short leaflets and triangular segments. In the case of *C. euxina* samples, perennial taxa, the leaves were observed not to be pinnate, displaying a more dentate fragmentation (Figure 2J). Additionally, the densely pubescent hairs of its indumentum were identified as a diagnostic characteristic in habitus. *C. altissima* leaves exhibited 3-pinnatisect fragmentation, which has been recognized as an important criterion for distinguishing it from *C. austriaca* in most genotypes (Figure 2G and 2H). Following morphological analysis of particular *Tripleurospermum* samples, it has become clear that identifying this genus based solely on leaf morphology poses challenges. Minor variations in leaf morphology have been noted among different taxa, with *T. tenuifolium* displaying a fringed (fimbriated) appearance and *T. baytopianum* exhibiting sparse fragmentation. Remarkably, the leaflets of *T. baytopianum* and *T. hygrophilum* samples demonstrate fragmentation in a spatulate form. The study revealed notable variations in the size of the basal and middle leaves of *Tanacetum* samples compared to the other three genera (Figure 2M and 2N). It was observed that *Tanacetum* specimens exhibit diversity in leaf morphology within the genus. Furthermore, their disintegration was more evident in comparison to other taxa, with *T. parthenium* samples displaying notably extensive and mainly pinnatisect fragments.

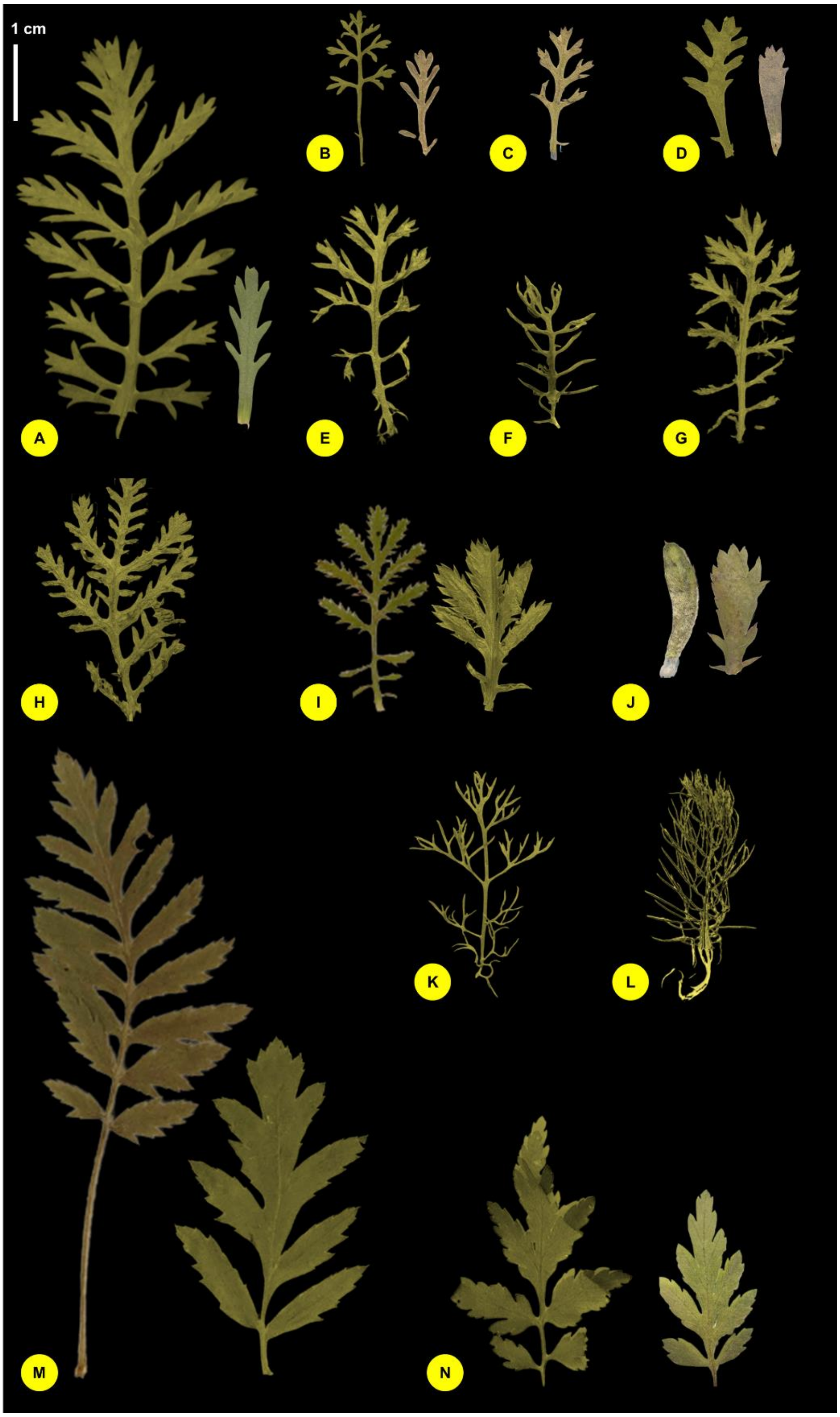

Figure 2. Leaves form of some Anthemidinae taxa. A- *A. arvensis* (b, u); B- *A. chia* (m, u); C- *A. pseudocotula* (m); D- *A. tomentosa* (m, u); E- *A. cotula* (m); F- *A. pectinata* var. *pectinata* (m); G- *C. altissima* (m); H- *C. austriaca* (m); I- *C. tinctoria* var. *tinctoria* and *C. tinctoria* var. *pallida* (m); J- *C. euxina* (u, m); K- *T. decipiens* (m); L- *T. tenuifolium* (b); M- *T. corymbosum* subsp. *cinereum* (b, m); N- *T. parthenium* (m, u). b; basal, m; middle, u; upper. Scale bar: 1 cm.

Table 5. Diagnostic morphological features of middle leaves in Anthemidiniae taxa.

Taxon*	Leaf Shape	Leaf Size (cm)	Fragmentation	Leaflet Number	Leaflet Shape	Apex
Aarv	Oblong, oblanceolate or obovoid	2.1-3 x 1-1.5	2-pinnatisect	6-9 or 9-13	Linear-oblanceolate	Acute
Aaur	Oblong-oblanceolate	3-6 x 0.5-1	2-3-pinnatisect	7-9	Oblanceolate, subulate-oblong	Acute
Achi	Oblong-oblanceolate	2.5-7 x 1-2	2-pinnatisect	6	Oblanceolate	Acute
Acot	Obovoid	2-5 x 1-3.25	2-3-pinnatisect	7-11	Linear-oblanceolate	Acute
Acre	Subulate, eliptical-obovoid	2-4 x 0.5-1	Distant	N/A	N/A	Acute
Apec	Oblong-lanceolate	0.5-2 x 0.35-0.85	Pectinate pinnate	7-15	Linear	Spiny acuminate
Apse	Oblanceolate	1.5-3	2-3-pinnatisect	N/A	Oblanceolate	Acute
Atom	Ovoid-oblong or oblong-subulate	2-5 x 0.3-1	Simple, dentate, 1-2-pinnatisect	6-7	Oblong	Obtuse
Calt	Elliptical, obovoid to oblanceolate	3.5-7 x 2.5-4.5	3-pinnatisect, 2-pinnatisect	7-14	Oblong-elliptical	Acute-acuminate
Caus	Oblanceolate	2.75-7.5 x 1.7-3	2-pinnatisect	7-15	Oblong to elliptical-oblanceolate	Acute
Ceux	Obovoid to oblong-oblanceolate	1-1.85 x 0.4-0.8	1-pinnatisect	11	Serrate, triangular	Acute
Ctin	Oblanceolate to oblong	3-3.75 x 1.5-2	2-pinnatisect	5-13	Linear-oblong	Acute
Cpal	Oblanceolate	3-4 x 1.2-2	2-pinnatisect	7-13	Oblong	Spiny acute
Tcor	Serrate-dentate, elliptical-linear	5-11.5 x 2-5.5	Pinnatisect	9-20	Elliptical-oblong	Spiny acuminate
Tpar	Obovoid	5 x 3	1-2-pinnatisect	7	Oblong	Acute
Tbay	Spatulate-obovoid	4 x 1.35	Laciniate, 2-pinnatisect	7	Linear	Moucronate
Tcon	Linear-lanceolate	N/A	2-3-pinnatisect	N/A	N/A	Moucronate
Tdec	Oblanceolate-elliptical	3.5 x 2	2-pinnatisect	7-12	Aristate-lanceolate	Aciculate
Thyg	Oblanceolate-ovoid	3-4 x 2	Filiform, 1-2-pinnatisect	7-10	Subulate	Acute-moucronate
Tten	Oblong-elliptical	4.5-7 x 2-4	2-3-pinnatisect	10-12	Aristate-subulate	Aciculate

*For taxon list see Table 1.

The mature achene morphology in *Anthemis* samples is considered a key criterion for diagnosis, in addition to other diagnostic characteristics (Figure 3, Table 6). For instance, while *A. arvensis* achenes exhibit a rib structure, they are also distinguished by a rimmed upper part. Furthermore, the auricle structure of *A. auriculata* specimens is notably distinctive, and dimorphism is observed in the achenes of these species, with the auricle structure being either membrane or opaque in the middle and lateral achenes (Figure 3A). Leaf morphology also serves as a distinguishing factor, particularly in comparison to *A. tomentosa*, a similar species concerning auricle (Figure 3G). Additionally, *A. auriculata* was observed to have a more oblong leaf structure. In terms of rib structure, *A. pseudocotula* taxa may be mistaken for *A. arvensis* (Figure 3B and 3F), yet they could be distinguished by the linear paleae and the presence of the capitulum of the paleae only in the middle part, not at the margins. Conversely, *A. cotula* samples are characterized by their indistinct rib and tubercled tissue, as well as the absence of a corona structure, which sets them apart from *A. pectinata* achenes (Figure 3C and 3E). Notably, the leaf morphology of *A. pectinata* specimens and the discoid capitulum serve as distinctive features for this species. Moreover, achenes belonging to the *umbilicata* taxon are characterized by their shiny surface, square appearance in cross-section, and sulcate nature. Differences in the achene morphology of the *Cota* genus are utilized as a distinctive characteristic for identifying various species (Figure 3H-L). For instance, the number of ribs and the shape of the achenes in the annual *Cota* species *C. altissima* and *C. austriaca* exhibit notable variances. In the *C. altissima* taxon, a distinct feature is the presence of 10 or more ribs on both surfaces, with the middle rib appearing carinate, giving the achene a diamond-like cross-sectional appearance. Conversely, the *C. austriaca* species is characterized by having 3 or fewer ribs, with a relatively longer corona. Meanwhile, the perennial *Cota* species, *C. euxina*, can be differentiated from others based on their leaf morphology, capitulum structure, and achene characteristics. Similarly, within the *C. tinctoria* species, rib shape serves as a distinguishing feature. Additionally, *C. tinctoria* var. *tinctoria* and *C. tinctoria* var. *pallida* taxa are identified based on ligula color among the *C. tinctoria* varieties. *Tripleurospermum* taxa are identified by the presence of glands and three prominent ribs on the back, setting it apart from other Anthemidinae taxa. *T. baytopianum* samples exhibit achenes with marginal coronas, while *T. hygrophilum* showcases longer coronas and thicker, oblong achenes. The *T. decipiens* taxon is recognized by its large size and extensive branching, along with rugose front surfaces on the achenes. In contrast, *T. tenuifolium* and *T. conoclinium* could be distinguished by the color of their mature achenes, which may range from dark to light. These taxa also exhibit a reduction in the glands at the top of the achene as it matures. Notably, the glands in *Tripleurospermum* fruits are visible even in immature achenes, serving as a distinguishing feature from the *Tanacetum* genus and can be identified through the examination of immature achenes.

Figure 3. Achenes immature /+ mature form of some Anthemidinae taxa. A- *A. auriculata*; B- *A. arvensis*; C- *A. pectinata* var. *pectinata*; D- *A. cretica* subsp. umbilicata; E- *A. cotula*; F- *A. pseudocotula*; G- *A. tomentosa* var. *tomentosa*; H- *C. altissima*; I- *C. austriaca*; J- *C. euxina*; K- *C. tinctoria* var. *pallida*; L- *C. tinctoria* var. tinctoria; M- *T. baytopianum*; N- *T. conoclinium;* O- T. *decipiens* (m); P- *T. hygrophium*; R- *T. tenuifolium*. Scale bar: 1 mm.

Table 6. Diagnostic morphological features of achenes in Anthemidiniae taxa.

Taxon*	Shape	Size	Rib	Apex	Gland	Notes
Aarv	Square	2 mm	Present	Rimmed	-	Naked tissue
Aaur	Circular	1.8-3 mm	8-10	1 mm auricle	-	Ribbed
Achi	Obconical	2-2.5 mm	N/A	Rimmed	-	Scarious auricle
Acot	Turbinate	1.35-1,5 mm	Non obvious	Round	-	Tuberculate tissue, brownish in mature
Acre	Square	1.5-2 mm	Sulcate	Flat	-	Bright tissue, brownish
Apec	Obconical	1.75-2 mm	8	Corona	-	Yellowish brown
Apse	Obconical or prismatic	1.25 mm	10	Shortly	-	Persistent in mature
Atom	Obconical	2 mm	Flat	Auricle	-	Light to dark brownish
Calt	Cuneate	2.25-2.5 mm	20	Corona	-	Compressed
Caus	Oblong-cuneate	2-2.75 mm	4 or 6	Undulate	-	Winged-like margin
Ceux	Oblong	1.65-2 mm	Present	0.25 mm corona	-	Compressed
Ctin	Pressed	1.75-2 mm	Thin	0.25 mm corona	-	Glabrescent tissue
Cpal	Rhomboid	1.85-2 mm	Non obvious	Corona	-	Glabrescent tissue
Tcor	Circular	2.1 mm	Present	Corona	-	Brownish
Tpar	Oblong-circular	1-1.5 mm	Whitish	Irregular	-	Grey-like tissue
Tbay	Compressed	1.5-2 mm	3 obvious	Corona	+	Mucilage
Tcon	Obovoid to oblong	1-2 mm	3 obvious	Non coronate	+	Apexial glandulate
Tdec	Oblong	1.5 mm	3 ribbed	Corona	+	Glabrescent
Thyg	Oblong	1.5 mm	3 ribbed	½ to ¼ corona	+	Non obvious ribbed
Tten	Compressed	1-2 mm	3 ribbed	Non coronate	+	Whitish in mature

*For taxon list see Table 1.

The classification of Anthemideae subtribes initially relied on morphological characteristics (Bremer and Humphries, 1993). However, subsequent molecular phylogenetic studies revealed that many of these subtribes were not monophyletic under this classification method. Consequently, a new subtribal classification using barcoding sequences was proposed. Notably, an analysis of chloroplast DNA sequences (ndhF, trnL-trnF) resulted in the monophyletic separation of *Anthemis* from *Tripleurospermum* and *Cota*, indicating a shared ancestral lineage between the latter two, while *Tanacetum* appeared paraphyletic. Additionally, a dendrogram based on ribosomal DNA sequences (ITS1, 5.8S, ITS2) revealed the separation of *Anthemis* and *Tripleurospermum* from a common ancestral lineage, with *Cota* and *Tanacetum* displaying paraphyletic characteristics. Furthermore, the study highlighted the close relationship between the Anthemidinae and Matricariinae subspecies. Nevertheless, the mutual monophyly within these groups remained subject to interpretation (Oberprieler et al., 2006, 2007, 2009, 2022).

The capitulum structures exhibits variation attributable to receptacle architectures, the presence or absence, and the morphologies of individual floret types, as well as the aggregation of capitula, contributing to the formation of higher order configurations (Fu et al., 2023; Zhang et al., 2021). The involucre, a common feature in all plant species of the Asteraceae family, plays a vital role as a protective structure. It encompasses a range of protective features such as secretory or non-secretory trichomes and appendages like spines, all of which contribute to shielding the plant from various environmental and biological threats. The involucre essentially serves as a multi-functional defense mechanism, enhancing the plant's ability to thrive in its ecosystem (Villagra et al., 2014). A cursory description of the capitulum, such as only indicating its diameter or the quantity of ray florets, appears to be insufficient and this has been proposed to lead to a lack of morphological uniformity in the capitulum (Fu et al., 2023). Additionally, it has been noted that this approach may overlook more nuanced yet crucial differences in other characteristics (Torices and Méndez, 2011; Fu et al., 2023).

One of the distinguishing features of *Anthemis* taxa compared to other members of the Anthemidinae group is the presence of paleae. Not all Anthemidinae taxa have these receptacular bracts, making this feature consistent at the genus level (Sell and Murrell, 2006; Skilbeck et al., 2019; Stace, 2019). Due to these distinct characteristics, it is advisable to continue utilizing paleae as a diagnostic feature. Notably, this structure is absent in *Tripleurospermum* and the *Matricaria* taxa, which are often mistaken for *Anthemis*. *Anthemis* taxa are categorized as either perennial or annual in the initial stage of the diagnostic key, as outlined in Davis' Flora of Turkey (1975). However, this classification may not always offer a clear distinction. In addition to growth form characteristics, employing a key that incorporates leaf fragmentation (a distinguishing feature for perennials), phyllary morphology, and paleae characters can help resolve this issue. When comparing the appearance of *A. cotula* and *A. pseudocotula*, the leaf morphology may resemble that of *M. chamomilla* var. *recutita*. These examples (*A. pseudocotula* and *A. cotula*), categorized as Maruta in Davis's Flora series, can be distinguished by the absence of paleae at the margins of the receptacle, a predominantly central location, and a linear shape. While the bracts of *A. arvensis* are rectangular-mucronate, they exhibit a linear-lanceolate-pointed morphology in *A. cotula* taxa. In *A. auriculata*, the marginal and middle achenes may be dimorphic, with the auricles being either opaque or membranous. In a study carried out by Ali (2019) using internal spacer regions (ITS) transcribed from nuclear ribosomal DNA (nrDNA), it was discovered that *A. cotula* and *A. pseudocotula* formed a monophyletic group in the NJ phylogram, whereas *A. arvensis* was found to be paraphyletic (Ali, 2019). The molecular study data has led to the conclusion that the paleae feature, along with other morphological criteria, can significantly contribute not only to the differentiation between genera but also to the distinction between species within the same genus.

Based on this current research concerning achenes, it is evident that there exists a greater phenotypic variation among species of the *Anthemis* genus when compared to others. This variability is notably apparent in the diverse rib structures. For example, achenes of *A. cretica* display sulcation, while those of *A. arvensis* and *A. pseudocotula* present ribbed structures. *A. auriculata* and *A. tomentosa* feature auricle structures, whereas *A. cotula* and *A. pectinata* taxa exhibit tuberculated achenes, with *A. cotula* displaying a dark coloration when mature. While *Cota* species may not demonstrate as much diversity as *Anthemis*, they exhibit distinct features within their group. The analyzed samples consistently revealed corona and rib structures. Additionally, the mature achene color and the number of ribs can serve as distinguishing factors between species and subspecies. The distinguishing characteristics of *Tripleurospermum* and *Cota* achenes are crucial for identifying the genera, but there are still inconsistencies and confusion in species differentiation. Conversely, *Anthemis* achenes exhibit greater variability.

In previous studies, it has been noted that *Tripleurospermum* can be morphologically mistaken for taxa such as *Anthemis* and *Matricaria* (Kay, 1976; Inceer et al., 2018). In line with our findings, it has been observed that fundamental characteristics can be utilized for distinguishing this genus morphologically (Inceer et al., 2012). For the *Tripleurospermum* genus, various researchers have highlighted criteria such as achene shape, length, corona structure, number of ribs, thickness, and the presence or absence of glands (Enayet-Hossain, 1975; Inceer and Ozcan, 2021; Skilbeck et al., 2019). Nevertheless, similar morphological features in taxa of the same genus can pose challenges to the identification process. The DNA barcoding method, increasingly recognized as a valuable tool for plant identification, may offer a solution to this issue (Zhang and Jiang, 2019). The nrDNA ITS has been frequently employed as a barcoding technique. Successful results have been reported by integrating these methods with other molecular techniques and morphological data (Kress et al., 2005; Chen et al., 2010; Kress, 2017).

Conclusion

The findings of our study revealed that morphological characteristics are effective in distinguishing the genera of the Anthemidinae subtribe. However, specific attention should be given to sub-genus categorization. Remarkable variations in achenes aid in identifying the genus *Anthemis*, while the paleae structure in the genus *Cota* is crucial in distinguishing annuals. Leaf morphology is sufficient for diagnosing *Tanacetum*, and mature achenes are necessary for characterizing the *Tripleurospermum* genus. It is essential for the diagnostic characters used in morphological identification to be supported by molecular-level characterization studies, genotyping, and DNA fingerprint analysis to accurately determine phylogenetic relationships and evolutionary lineages.

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