

TAXONOMIC SIGNIFICANCE OF CYPSELAS MORPHOLOGY OF TRIBE HELIANTHEAE (ASTERACEAE) FROM PAKISTAN AND KASHMIR

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In Asteraceae, the remarkable feature of cypselas plays a significant role in the taxonomic treatment of species. In this research, we aim to assess the significance of the micro-morphological diversity of cypselas at a lower taxonomic level, alongside its corresponding phylogeny. Cypselas of 11 taxa viz., *Blainvillea acmella* (L.) Philip., *Eclipta prostrata* (L.) L., *Helianthus annuus* L., *Helianthus tuberosus* L., *Parthenium hysterophorus* L., *Sphagneticola calendulaceae* (L.) Pruski, *Sphagneticola trilobata* (L.) Pruski, *Verbesina encelioides* (Cav.) Benth. & Hook. f. ex A. Gray, *Xanthium spinosum* L., *Xanthium strumarium* L., and *Zinnia elegans* Jacquin were examined for the first time from the study area. Cypselas are usually isomorphic (except in *Zinnia elegans*), light to dark brown or blackish with a carbonized layer in the pericarp, often monotypic, oblanceolate to oblong or obovate. The characters including the size, surface, number of ribs, angle, apex, and appearance of awns, pappus, beak, and wings were evaluated. Studied taxa are grouped into two contrasting cypselas types (viz., Type I and Type II). Cypselas types with their species are presented through the artificial key. Thirteen qualitative and quantitative variables were utilized to construct cluster analysis, including hilum position, degree of pubescence (surface hair), apex, presence or absence of pappus and awns. The variations among these features were the cornerstone to delineating studied taxa. Here we report the cypselas morphological characteristics of 11 species from 8 genera of Heliantheae (Asteraceae) for the first time in Pakistan and Kashmir, using Stereomicroscopy and Scanning Electron Microscopy.

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Keywords: awns; cypselas morphology; Heliantheae; Kashmir; Pakistan; pappus

ارزش تاکسونومیکی صفات ریختی فندقه‌ها در قبیله **Heliantheae** از خانواده **Asteraceae** از پاکستان و کشمیر

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در خانواده Asteraceae، صفات فندقه جایگاه قابل توجهی در طبقه‌بندی ایفا می‌کند. در کار تحقیقاتی حاضر، خصوصیات فندقه و همچنین ارزیابی ارزش تنوع میکرومورفولوژیکی ریزریخت‌شناسی آن در طبقه‌بندی در سطوح تاکسونومیکی پایین‌تر بررسی می‌شود.

فندقه ۱۱ آرایه (*Blainvillea acmella* (L.) Philip., *Eclipta prostrata* (L.) L., *Helianthus annuus* L., *Helianthus tuberosus* L., *Parthenium hysterophorus* L., *Sphagneticola calendulaceae* (L.) Pruski, *Sphagneticola trilobata* (L.) Pruski, *Verbesina encelioides* (Cav.) Benth. & Hook. F. ex A. Grey, *Xanthium spinosum* L., *Xanthium strumarium* L., and *Zinnia elegans* Jacquin) برای اولین بار از منطقه مورد بررسی مطالعه شدند. فندقه‌ها معمولاً هم شکل هستند (به جز در *Zinnia elegans*)

(Zinnia)، قهوه‌ای روشن تا تیره یا مایل به سیاه با لایه کربنی در پریکارپ، اغلب یکنواخت و به شکل‌های، واژسرنیزه‌ای، مستطیلی و یا واژتخم مرغی هستند. اندازه، سطح، تعداد رگه‌ها، زاویه، راس و شکل‌های شاخک‌ها و پرزها، منقار و بال‌ها، صفات مورد بررسی بودند. گونه‌های مورد مطالعه در دو نوع متفاوت (نوع I و نوع II) طبقه‌بندی و انواع فندقه‌ها و گونه‌های متعلق به آنها توسط یک کلید شناسایی از یکدیگر تفکیک شدند. ۱۳ متغیر کمی و کیفی برای انجام آنالیز خوشه‌ای مورد استفاده قرار گرفتند از جمله موقعیت ناف، میزان کرک‌دار بودن (موی سطحی)، راس، وجود یا عدم وجود جقه و شاخک. تغییرات بین این ویژگی‌ها مبنای تفکیک گونه‌های مورد مطالعه بوده است. در این مقاله خصوصیات ریخت شناسی فندقه در ۱۱ گونه از ۸ جنس از قبیله Asteraceae (Heliantheae) برای اولین بار، با استفاده از استرئومیکروسکوپ و میکروسکوپ الکترونی برای گونه‌های پاکستان و کشمیر مورد بررسی قرار گرفته است.

INTRODUCTION

Heliantheae is the most morphologically extended tribe, with 113 genera and 1500 species, distributed worldwide. The members of this group disperse widely, spanning from pantropical regions to the arid plains of both tropical and temperate zones across the globe (Panero, 2007b). Tribe Heliantheae is commonly found in northern areas of Pakistan. The distribution extended from district Chitral, Gilgit, and Kashmir to plains and mountainous areas of Baluchistan (Zhob, Ziarat, Quetta, Khuzdar) and Sindh (Larkana, Khairpur, Dadu, Karachi, Thatta and Hyderabad) (Perveen & Qaiser, 2021).

Cassini (1819) was the first to comprehensively describe this tribe with the formal title of "Helianthees". Later Bentham (1873) originated the framework of classification of Heliantheae. Some earlier authors (Clarke 1876; Davis 1975; Heywood & al. 1976; Dassanayake & al. 1980; Hook, 1881; Borivosa & al. 1990; Chen & Hind 2011), proposed the taxonomic treatment of Heliantheae and its allied tribes in a broader sense that further influenced their nomenclatural conflicts. In the 20th century, Heliantheae underwent molecular phylogenetic studies to provide valuable evidence for the decision of generic circumscription (Panero 2007a & b; Baldwin 2009). Based on this evidence, Perveen & Qaiser (2021) followed the treatment of Jeffrey (Kadereit & Jeffrey 2007) and accepted Heliantheae as a distinct tribe with 12 genera presenting 17 species from Pakistan and Kashmir. The tribe is characterized by isomorphic cypselas with carbonized walls, compressed or rarely obcompressed, biconvex, narrowly obovate to sub-orbicular in outline (Perveen & Qaiser 2021).

Cypselas morphological characteristics of Heliantheae have valuable support in phylogeny and systematics for instance, Robinson (1981) expanded the tribe Heliantheae into 35 subtribes and recognized them based on structural and chemical studies. The

main characteristics viz., resin duct pattern, the presence of fiber-sheaths in corollas disk, and patterns of striations in achenes were found more advanced than in the other tribes. Similarly, Panero (2007a & b) referred to paleate receptacle and carbonized cypselas (presence of phytomelanin layer in the cypselas) as significant features of the tribe Heliantheae based on chloroplast DNA sequencing.

Many taxonomists presented extensive literature based on the cypselas morphological studies related to various tribes of Asteraceae such as Blanca & Guardia (1997), Mukherjee & Sarkar (2001), Garg & Sharma (2007), Marzinek & Oliveria (2010), Roque & Funk (2013), Karaismailoglu (2015), Angulo & al. (2015) and Silva & al. (2018). Similarly, from Pakistan and Kashmir reports on the cypselas morphology are Inuleae (Abid & Zehra 2007), Gnaphalieae (Abid & Qaiser 2007), Anthemideae (Abid & Qaiser 2009), Senecioneae (Abid & Ali 2010), Mustisieae (Abid & Alam 2011), Cichorieae (Abid & Qaiser 2015; Sana & al. 2020; Abid & al. 2022), Coreopsideae (Akhlaq & al. 2022). Bona & al. (2023) studied the macro and micro-morphological characters of 15 species from the genus *Centaurea* L. They found significant variations in shape, size, surface pattern, and type of pappus as markers for identification at infrageneric ranking among selected species. Karodpati & Dhabe (2013) studied cypselas characters of 16 Asteraceae taxa and concluded that Heliantheae cypselas are highly variable. For instance, dimorphic cypselas, presence of awns, palea, wings, and pappus were found significant in decisions of taxonomic ranking at generic and specific levels. Besides these researches, several studies have emphasized the high taxonomic value of fruit and seed characteristics of different tribes and families from the overall world (Khajehpiri & al. 2010; Hoseini & al. 2017; Arabi & al 2017) however, no comprehensive investigation carried out from Pakistan, Therefore, the cypselas morphology of 11

examined Heliantheae species is undergoing investigation for the first time from Pakistan and Kashmir. The aims of the present study are: (a) to determine the taxonomic significance of cypselas morphology both at the generic and species level by numerical analysis. (b) to find a correlation between cypselas morphology and gross morphology. (c) to evaluate the role of cypselas morphology in the systematics and phylogeny of the tribe.

MATERIALS AND METHODS

Mature cypselas of 11 species belonging to 8 genera namely *Blainvillea*, *Eclipta*, *Helianthus*, *Parthenium*, *Sphagneticola*, *Verbesina*, *Xanthium*, and *Zinnia* were examined for morphological studies. The cypselas materials of selected taxa were mostly collected from fresh specimens taken during field surveys and then voucher specimens submitted to Karachi University Herbarium. Whereas, some cypselas material is collected from Herbarium material (KUH), Table 1. Various parameters including size, shape, color, surface texture, and presence of awns, beaks, wings, and pappus were observed under stereomicroscope (Nikon Type 102). Other observations related to micro characters including carpogonium and foramen were examined under a

scanning electron microscope (Joel JSM-6380A). A supplementary step was followed before Scanning the electron microscope. The selected specimens were mounted on a metallic stub, using double adhesive tape. Stubs were placed in the sputtering chamber for 6 minutes to be coated with gold and then observed under the Scanning electron microscope. The descriptive terminology used here is based on Streat (1992) and Roque & al. (2009).

For the numerical analysis, dendrogram formulation is formulated by using the Euclidean distance as the resemblance characteristics and Ward's method to build the close lineage between the members of the selected tribe. The evaluation is based on contrasting characters of cypselas to find the correlation among the studied species of Heliantheae. The computations were done using the SPSS software (IBM-SPSS statistical analysis software 21). A total of 13 characters were selected to distinguish the studied species. The qualitative characters are denoted by (0 and 1) to indicate the absence or presence of character, binary state (by 1 and 2) and multiple state (by 1, 2, and 3) to indicate the variation in character. The characters and character states used for hierarchical clustering are listed in Tables 2 & 3.

Table 1. List of voucher specimens examined for cypselas morphology.

Taxa	Collection data
<i>Blainvillea acmella</i>	Moin, Bushreen & Zeenat 41 (KUH); Abrar Hussain s.n (KUH); Abrar Hussain s.n (KUH); S.M.H.Jafri 4118 (KUH); A.Ghafoor & Tahir Ali 4163 (KUH)
<i>Eclipta prostrata</i>	Mr. Yasir Ahmed 47952 (KUH); Mr. Sadar uddin 47927 (KUH); Shameen Aisha 47956 (KUH); Kamal Akhtar & S. Nazim uddin 939 (KUH); Rabia Akhlaq & Taba Rauf 95844 (KUH); Rabia Akhlaq & Taba Rauf s.n (KUH); Rabia Akhlaq & Taba Rauf s.n (KUH)
<i>Helianthus annuus</i>	Moin, Zeenat & Bushreen 9 (KUH); Sadaruddin, Moin & Nadeem 108 (KUH); S.M.H.Jafri 1383 (KUH); Rabia Akhlaq & Taba Rauf 95832 (KUH); Rabia Akhlaq & Taba Rauf 95834 (KUH); Ali Noor 1713 (KUH); Zeenat & Bushreen 164 (KUH)
<i>Helianthus tuberosus</i>	Fazal Amin & Haider Ali 1120 (KUH); Shabir Ijaz 393, 395, 397, 3912 (KUH)
<i>Parthenium hysterophorus</i>	Sajjad Haider 664 (KUH); Shabir Ijaz 1011 (KUH); Shabir Ijaz 635 (KUH); M. Qaiser & Rizwan Yousuf 7743 (KUH); Abdul Ghafoor 5392 (KUH); M. Qaiser & Abdul Ghafoor 4984 (KUH); M. Qaiser & Rizwan Yousuf Hashmi 7935 (KUH)
<i>Sphagneticola calendulaceae</i>	Anjum Perveen 2195(KUH)
<i>Sphagneticola trilobata</i>	Taba Rauf 12 (KUH)
<i>Verbesina encelioides</i>	Rabia Akhlaq & Taba Rauf 95615 (KUH); Rabia Akhlaq 95616 (KUH); Rabia Akhlaq & Taba Rauf 95617 (KUH); Rabia Akhlaq 95617, 95618 (KUH); Zamarrud & Sultanul Abedin 787, 788 (KUH)
<i>Xanthium spinosum</i>	Zhob Appozai 05 (KUH); Kamran Ishaq Bahandikhel 06 (KUH)
<i>Xanthium strumarium</i>	Tahir Ali, S. Z. Husain & Gohar Khan 57827 (KUH); S. I. Ali, W. Sugong, Tahir Ali & Gohar Khan 3185 (KUH); Tahir Ali & Tufail Ahmed 1333 (KUH); A.Ghafoor & Tahir Ali 3512 (KUH); Yasir 93411 (KUH)
<i>Zinnia elegans</i>	M.Qaiser 276 (KUH); Zeenat A.Razzak & Moin A.Razzak 106 (KUH); Moin, Bushreen & Zeenat 5 (KUH); Rabia Akhlaq & Taba Rauf 95833 (KUH)

Table 2. Character States for Cluster Analysis.

1	Average Length (mm): 0.1-6.0 (1), 6.5-12.0 (2)
2	Average Breadth (mm): 0.1-2.0 (1), 2.1-4.0 (2)
3	Degree of Pubescence (Cypselas-hair present): Absent (0), Present (1)
4	Surface: Not muricate (0), Muricate (1)
5	Number of ribs: Absent (0), Present (1)
6	Angle: Triangular (1), Quadrangular (2)
7	Apex: Truncate (1), Dentate (2), Attenuate (3)
8	Position of hilum: Basal (1), Lateral (2)
9	Wings: Present (1), Absent (0)
10	Surface of wings: Glabrous (0), Hispid (1)
11	Beak: Present (1), Absent (0)
12	Pappus: Present (1), Absent (0)
13	Awns: Absent (0), Present (1)

Table 3. Data Matrix for cluster analysis for the tribe Heliantheae.

S. No.	Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13
1	<i>Blainvillea acemella</i>	2	2	0	1	1	1	1	1	0	0	0	0	1
2	<i>Eclipta prostrata</i>	1	1	0	1	1	1	1	1	1	1	0	0	1
3	<i>Helianthus annuus</i>	1	2	0	0	1	2	1	1	0	0	0	0	1
4	<i>Helianthus tuberosus</i>	1	1	0	0	1	2	1	1	0	0	0	0	1
5	<i>Parthenium hysterophorus</i>	1	1	0	0	1	1	2	2	0	0	0	0	0
6	<i>Sphagneticola calendulaceae</i>	1	2	0	0	0	2	1	1	0	0	0	1	0
7	<i>Sphagneticola trilobata</i>	1	1	0	0	0	1	1	1	0	0	0	1	0
8	<i>Verbesina encelioides</i>	1	2	1	0	1	1	2	1	1	1	0	0	1
9	<i>Xanthium spinosum</i>	2	1	0	0	1	1	3	2	0	0	1	0	0
10	<i>Xanthium strumarium</i>	2	2	0	1	1	1	3	2	0	0	1	0	0
11	<i>Zinnia elegans</i>	2	2	1	1	1	1	2	1	1	1	0	0	0

RESULT

General cypselas characters in the tribe Heliantheae

Cypselas varied in shape such as oblanceolate-lanceolate (*Blainvillea*, *Eclipta*, *Sphagneticola*, *Verbesina*, *Xanthium*, and *Zinnia*), oblong, elliptical-obovate or ovate (*Helianthus*, *Parthenium* and *Xanthium strumarium*), black, grayish black-darkened (dark gray), brown, golden brown-dark brown, sometimes grayish green, 2-13 mm long, 0.8-5 mm wide. Usually, cypselas are smooth or glabrous but sometimes hairy (Scabrid) as in *Verbesina* and *Zinnia*. *Zinnia* showed a remarkable combination of hair with the occurrence of a muricate surface. A few genera also showed muricate surfaces such as *Blainvillea*, *Eclipta*, and *Xanthium*. The apex is

truncate (*Blainvillea*, *Eclipta*, *Helianthus*, and *Sphagneticola*), dentate (*Parthenium*, *Verbesina*, and *Zinnia*), attenuate (*Xanthium*); ribs present or rarely absent, 1-5 if present, 3 to 4-angular, compressed, triquetrous, hilum basal or lateral. Wings present or absent, 2 if present, 2-10 mm long, 0.3-4.5 mm wide, without hair or antrorsely hispid. Beak mostly absent (except in *Xanthium*) 1-2.5 mm long, smooth or antrorsely hispid. Awns present or absent, 2-3 if present, 0.5-4 mm long, glabrous to pubescent, barbed, antrorse (upward), sometimes scaly or slender, filiform (*Verbesina encelioides*) or fusiform (*Helianthus* spp.), entire or serrate, lime to pale yellow, sometimes light to dark brown. Pappus present or absent, uniseriate, 5-8 if present, rarely green, dull to pale yellow, 1.5-2 mm long, scaly

(*Sphagneticola calendulaceae*), fused and crown-like (*Sphagneticola trilobata*). Carpopodium distinct or indistinct, developed or undeveloped, with or without a complete ring, circular to elliptical or angular, symmetric or asymmetric, 84.1-771 μm in diameter, foramen distinct or indistinct, 42.7-413 μm in diameter, (Figs. 1-1 & 1-2).

Statistical Analysis

Figure 2 shows the hierarchical analysis based on 13 variables of cypselas of 11 species representing Heliantheae. The studied species appeared in separate clusters with short or long branches, indicating the variation among cypselas morphological characters. The presence or absence of awns, wings, beak, and pappus of cypselas, were found to be significant characters for delimitation of studied taxa. The hierarchical analysis grouped the studied species into Cluster I and Cluster II based on the position of the hilum.

Cluster I (Cypselas Type I) comprised 8 species representing 72.72% of total taxa, characterized by the cypselas with basal position of the hilum. In contrast, Cluster II included 3 species (Cypselas Type II) representing 27.27% of total taxa characterized by the cypselas with lateral position of the hilum. Furthermore, Cluster I grouped into two i.e. subcluster IA and IB. IA is characterized by a truncate apex and smooth surface (without hair) and accommodates 2 species of *Helianthus* (*H. annuus* and *H. tuberosus*), *Blainvillea acmella*, *Eclipta prostrata* and 2 species of *Sphagneticola* (*S. calendulaceae* and *S. trilobata*). Whereas subcluster IB accommodated two species *Verbesina encelioides* and *Zinnia elegans*, characterized by dentate apex and hairy surface.

Cluster II (Cypselas Type II) comprised 3 species (*Xanthium spinosum*, *X. strumarium*, and *Parthenium hysterophorus*). The species of *Xanthium* were distinguished by the presence of seed burrs (rough bracts of seed) and beaked cypselas, whereas, *Parthenium* appeared separately due to the absence of seed burrs and beaked cypselas.

Based on the morphometric data and cluster analysis, all the studied species were grouped into two major cypselas types: cypselas type I and Cypselas type II.

Cypselas Type I-Helianthus type

Cypselas oblanceolate to lanceolate, oblong or obovate, glabrous rarely scabrid, triangular to quadrangular, apex truncate except in *Verbesina* basal

hilum. Rarely pappus present (*Sphagneticola*). Awn present. The genera included in Type I are *Blainvillea* (*Blainvillea acmella* (L.) Philip.); *Eclipta* (*Eclipta prostrata* (L.) L.), *Helianthus* (*Helianthus annuus* L. & *Helianthus tuberosus* L.); *Sphagneticola* (*Sphagneticola calendulacea* (L.) Pruski & *Sphagneticola trilobata* (L.) Pruski); *Verbesina* (*Verbesina encelioides* (Cav.) Benth. & Hook. f. ex A. Grey); *Zinnia* (*Zinnia elegans* Jacquin)

Key to species of cypselas Type I

1. Cypselas not hairy (glabrous), apex truncate 2
- Cypselas hairy (scabrid), apex dentate 7
2. Cypselas without pappus, ribs distinct 3
- Cypselas with pappus, ribs indistinct 6
3. Cypselas not muricate, quadrangular with 2-3 awns 4
- Cypselas muricate, triangular with 2 brown awns 5
4. Cypselas obovate. Awns antrorsely barbed, entire *Helianthus annuus*
- Cypselas oblong. Awns glabrous, serrate *Helianthus tuberosus*
5. Cypselas 5-8 mm long, wingless. Awns antrorsely barbed *Blainvillea acmella*
- Cypselas 2-2.5 mm long, winged. Awns glabrous *Eclipta prostrata*
6. Cypselas lanceolate, quadrangular. Pappus free, scaly *Sphagneticola calendulaceae*
- Cypselas obovate, triangular. Pappus fused, crown-shaped *Sphagneticola trilobata*
7. Cypselas 5-7 mm long, not papillose. Awns present *Verbesina encelioides*
- Cypselas 6-9 mm long, papillose. Awns absent *Zinnia elegans*

Cypselas Type II - Xanthium type

Cypselas are obovate rarely lanceolate, glabrous, triangular, apex attenuate rarely dentate, hilum lateral. Beak present except in *Parthenium*.

The genera included in Type II are *Parthenium* (*Parthenium hysterophorus* L.); *Xanthium* (*Xanthium spinosum* L. & *Xanthium strumarium* L.)

Key to species of cypselas Type II

1. Cypselas 6-13 mm long, beaked 2
- Cypselas 2-3 mm long, not beaked *Parthenium hysterophorus*
2. Cypselas lanceolate, 6-7 mm long, up to 2 mm wide *Xanthium spinosum*
- Cypselas obovate, 9-13 mm long, up to 5 mm wide *Xanthium strumarium*

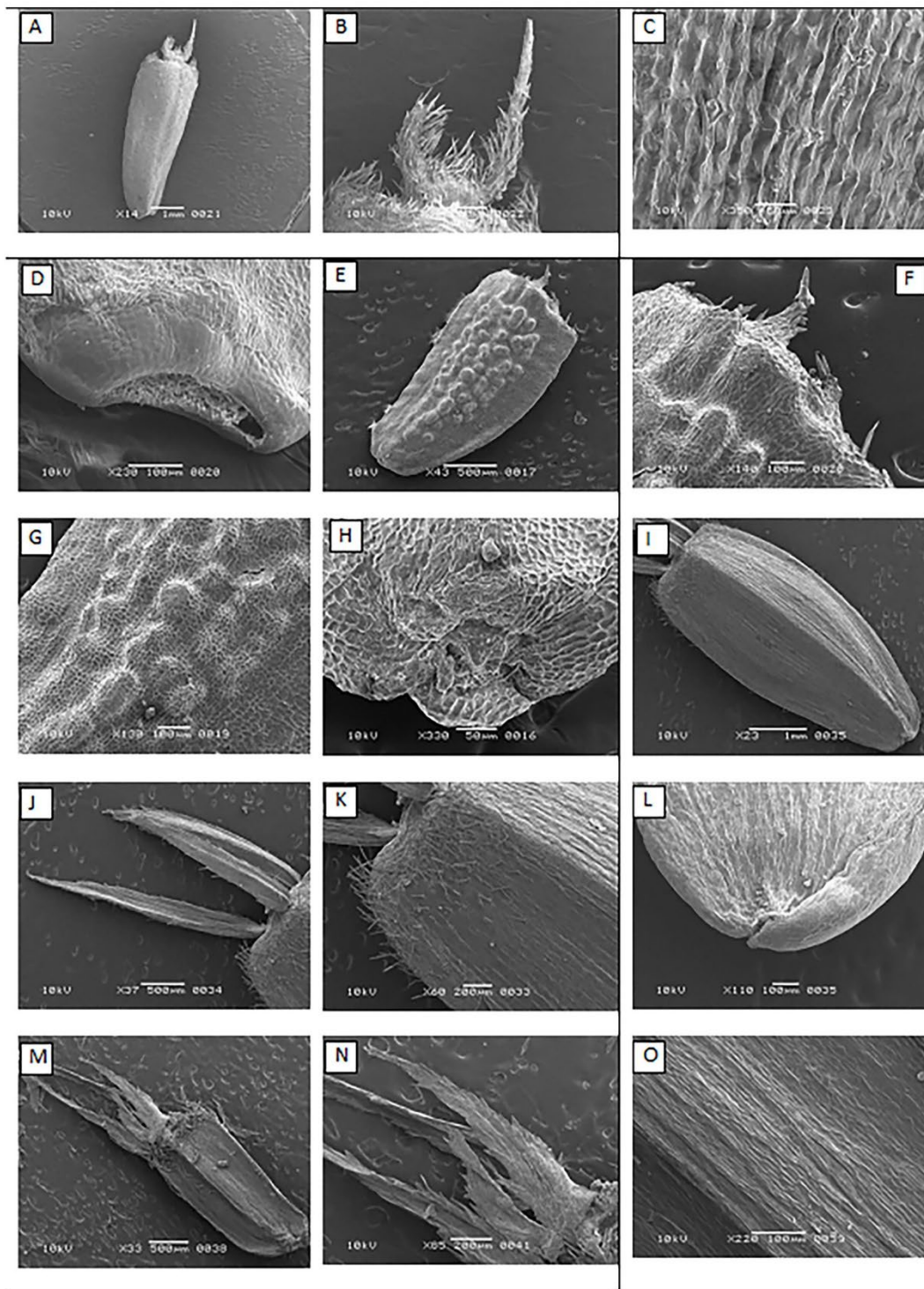


Fig. 1.1 Scanning Electron Micrographs (SEM) of the cypselas morphology: *Blainvillea acmella*: A, cypselas; B, awns; C, surface; D, carpodium. *Eclipta prostrata*: E, cypselas; F, awns; G, surface; H, carpodium. *Helianthus annuus*: I, cypselas; J, awns; K, surface; L, carpodium. *Helianthus tuberosus*: M, cypselas; N, awns; O, surface.

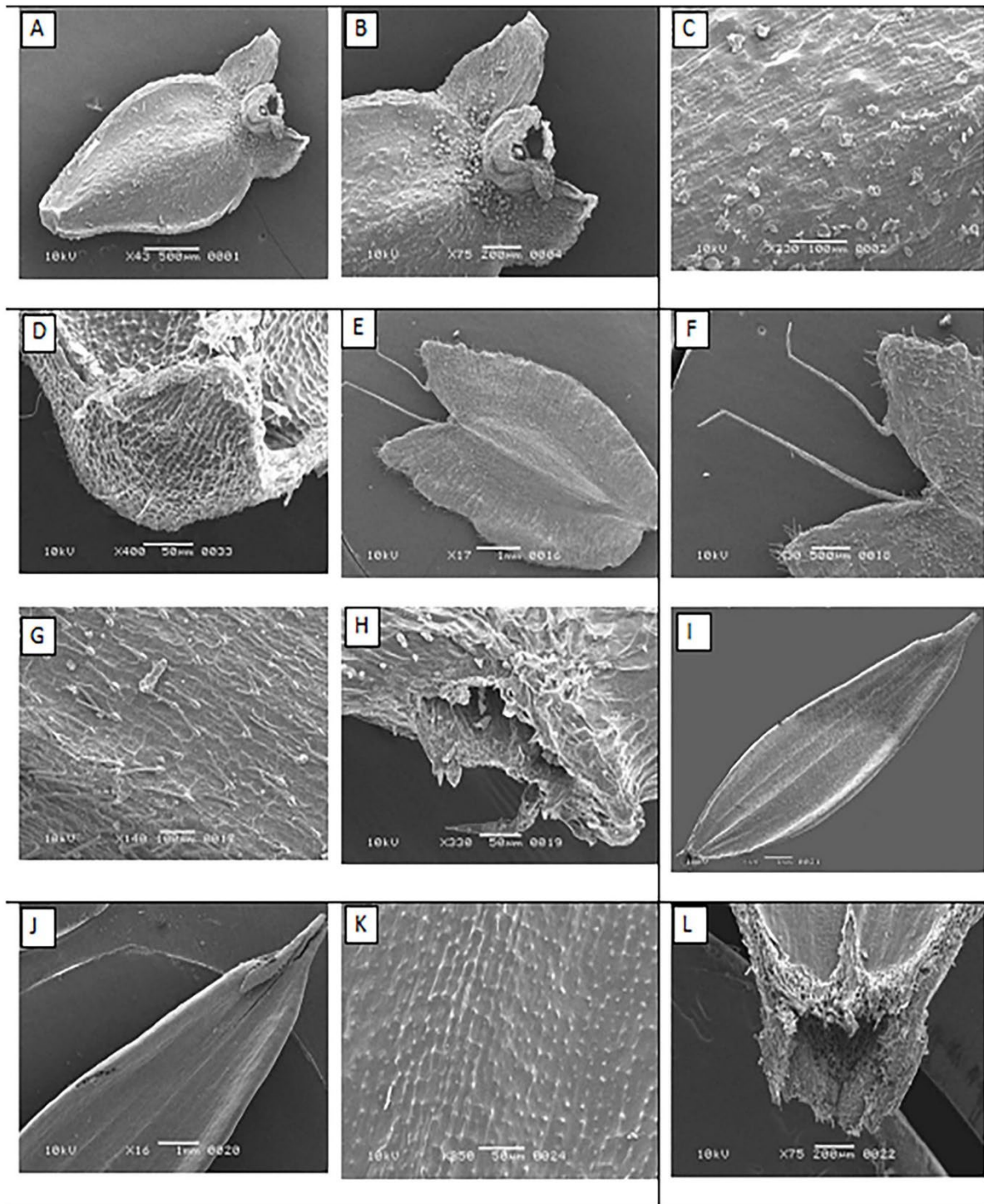


Fig. 1.2 Scanning Electron Micrographs (SEM) of the cypselas morphology: *Parthenium hysterophorus*: A, cypselas; B, apex; C, surface; D, carpodium. *Verbesina encelioides*: E, cypselas; F, awns; G, surface; H, carpodium. *Xanthium strumarium*: I, cypselas; J, awns; K, surface; L, carpodium.

DISCUSSION

The present research on Heliantheae showed that cypselas are monomorphic throughout the tribe, but variations in color and shape have been observed. The other qualitative and quantitative features have shown variations in cypselas size, degree of pubescence, number of ribs, position of hilum, and presence or absence of pappus, wings, beak, and awns (Table 4. & Figs. 3 & 4). Corresponding to the findings of earlier authors such as Panero (2007b); Garg & Sharma (2007); Baldwin (2009); Bona (2014); Frangiote & de-Souza (2014); Jana & Mukherjee (2014), Ozcan & Akinci (2019) and Akhlaq & al. (2022) the present study resulted that cypselas morphological characters are taxonomically valuable at the generic and specific levels. Their implications become stronger along with

micro-morphological characters. For instance, Akhlaq & al. (2022) investigated 11 species belonging to 4 genera of Coreopsideae (Asteraceae) from Pakistan and Kashmir. They found that cypselas characters are also a valuable diagnostic feature for the delimitation of genera and species.

In the examined taxa cypselas were ribbed (except *Sphagneticola*). The angle of cypselas varies from triangular (*Blainvillea*, *Eclipta*, *Parthenium*, *Verbesina*, *Xanthium*, and *Zinnia*) to quadrangular (*Helianthus* and *Sphagneticola*). The position of the hilum is basal to lateral (*Parthenium* and *Xanthium*). Our results are in agreement with the results recorded by Garg & Sharma (2007), Panero (2007b), Baldwin (2009), and Jana & Mukherjee (2014).

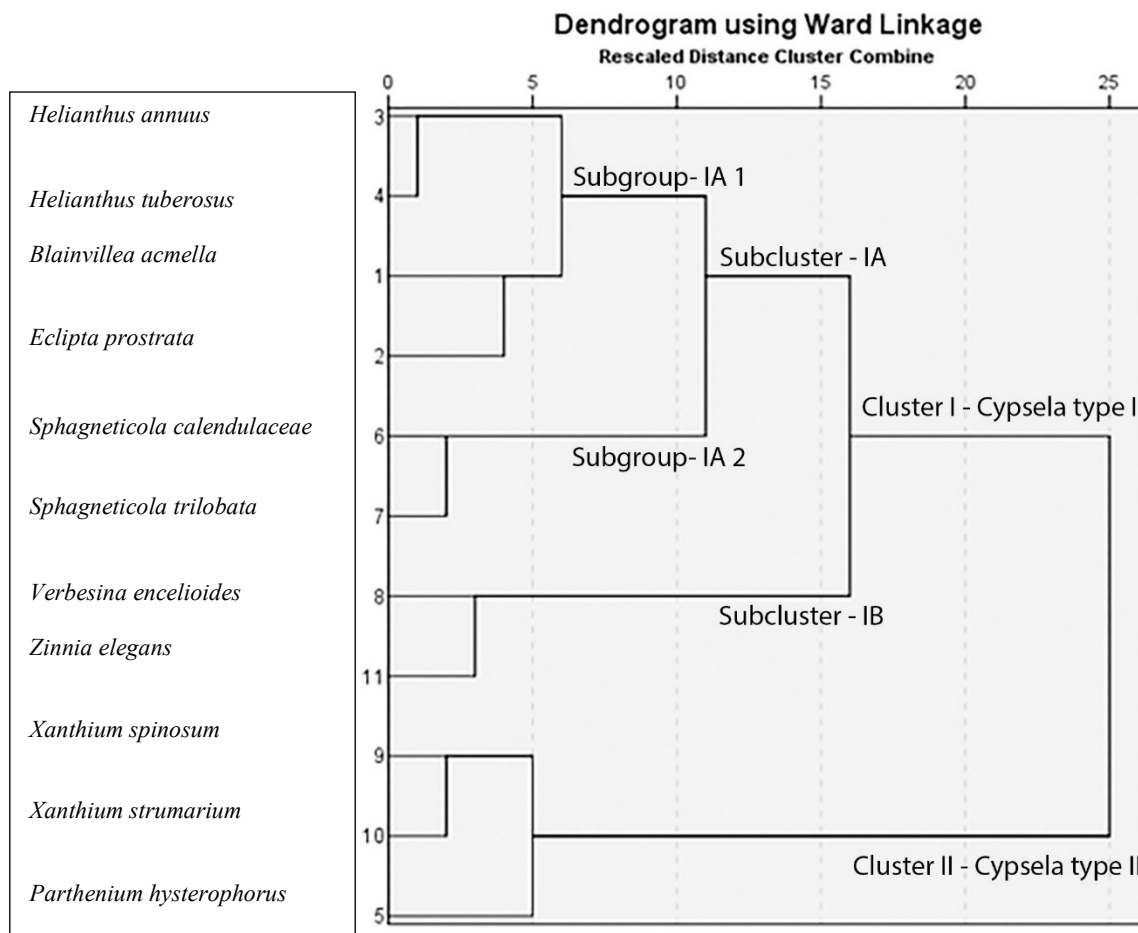


Fig. 2. Dendrogram showing the relationship of the species of tribe Heliantheae.

The surface texture and apex shape of cypselas are variable in the *Helianthus* type, for instance, cypselas surface glabrous and apex truncate in *Helianthus*, *Blainvillea*, *Eclipta*, and *Sphagneticola* (Fig.1, subcluster IA) and species are grouped. This subcluster further resolved into two subgroups viz., IA1 and IA2 by the absence and presence of pappus. *Sphagneticola* (IA2) is separated from the rest of the genera by having the pappus. In addition, *S. calendulaceae* and *S. trilobata* were significantly distinguished due to the size and shape of cypselas, and the pappus. *Helianthus*, *Blainvillea*, and *Eclipta* (IA1) are segregated due to the presence of awns instead of pappus. These findings did not support the results of the molecular studies of Panero (2007b), based on the chloroplast DNA sequencing. According to Panero (2007b); *Blainvillea*, *Eclipta*, and *Sphagneticola* belonged to the subtribe Ecliptinae whereas *Helianthus* belonged to the subtribe Helianthinae (tribe Heliantheae). Our results (Fig. 1) showed that *Sphagneticola* was fairly below the

Blainvillea and *Eclipta*. All three mentioned genera showed close affinities regarding cypselas morphology (dark brown to blackish, oblanceolate to lanceolate, triangular). *Helianthus* occupied an upright position in Fig. 1 (blackened, oblong to obovate, quadrangular). Furthermore, the shape of cypselas, surface texture of awn, and symmetry of carpodium were found significant in the segregation of *H. annuus* and *H. tuberosus*. Robinson (1981) placed the *Helianthus* in the subtribe Helianthinae based on achenes compressed, carbonized, and the presence of striations. Moreover, *Blainvillea* and *Eclipta* appeared as sisters in the same clade. This was supported by the findings of Garg & Sharma (2007) by showing a similar primary sculpture i.e. reticulate and considered both genera into the same subtribe Ecliptinae. However, it is interesting to note that morphologically both genera are keyed out in different groups based on paleae (covering of cypselas), (Perveen & Qaiser, 2021).

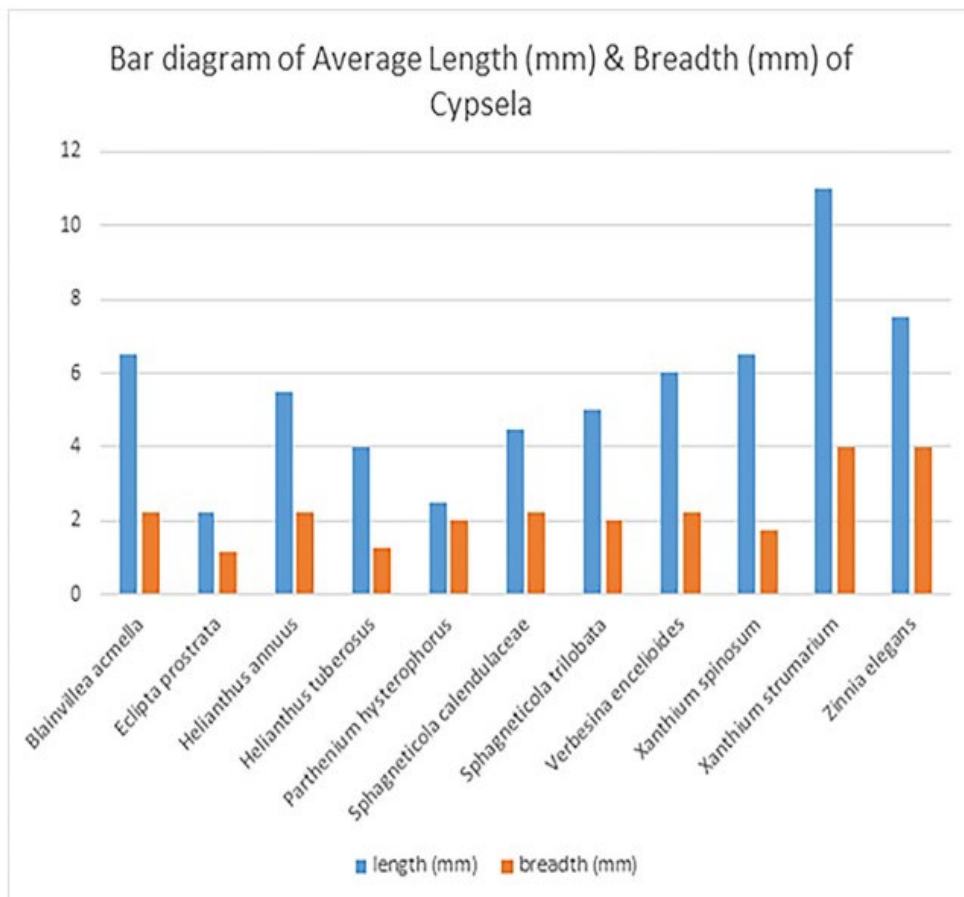


Fig. 3. Bar diagram represents the variation in average length and breadth (mm) of Cypselas in the tribe Heliantheae.

Pie chart showing the percentage of significant features of cypselas

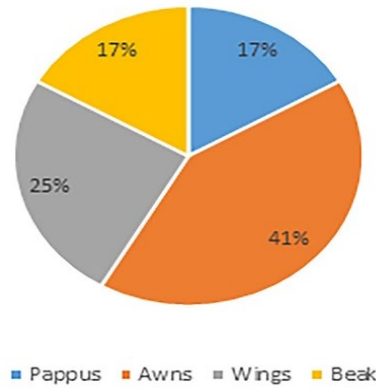


Fig. 4. Pie chart showing the percentage of significant features of cypselas in the studied species of the tribe Heliantheae.

Zinnia and *Verbesina* (Fig. 1, subcluster IB) showed separate positions because of the presence of hair on the surface of the cypselas and dentate apex. On the other hand, both genera are distinguished due to differences in the presence or absence of awns and surface texture of cypselas. Surprisingly, both genera are grouped separately based on the gross morphology of the corolla, ray floret, and blended cypselas (Perveen & Qaiser, 2021). Panero (2007b) placed *Zinnia* and

Verbesina into different subtribes viz., Zinninae and Verbesininae (tribe Heliantheae) respectively, based on the chloroplast DNA sequencing. According to Panero (2007b), Zinninae is characterized by epaleate receptacles, and black to stramineous cypselas without pappus whereas Verbesininae is characterized by paleate receptacles, black cypselas with pappus. Our results of *Zinnia* and *Verbesina* are in agreement with the phylogenetic studies of Panero (2007b).

Table 4. Comparison of cypselas diagnostic characters in different genera and species of tribe Heliantheae

Name of Taxa	Pappus	Awns	Wings	Beak
<i>Blainvillea acmella</i>	Absent	Present	Absent	Absent
<i>Eclipta prostrata</i>	Absent	Present	Present	Absent
<i>Helianthus annuus</i>	Absent	Present	Absent	Absent
<i>Helianthus tuberosus</i>	Absent	Present	Absent	Absent
<i>Parthenium hysterophorus</i>	Absent	Absent	Absent	Absent
<i>Sphagneticola calendulaceae</i>	Present	Absent	Absent	Absent
<i>Sphagneticola trilobata</i>	Present	Absent	Absent	Absent
<i>Verbesina encelioides</i>	Absent	Present	Present	Absent
<i>Xanthium spinosum</i>	Absent	Absent	Absent	Present
<i>Xanthium strumarium</i>	Absent	Absent	Absent	Present
<i>Zinnia elegans</i>	Absent	Absent	Present	Absent

In *Xanthium* type, cypselas morphology was comparatively identical, as they shared the features of lateral hilum and associated paleae covering i.e. cypselas associated with the subtending phyllary/burrs.

Both genera are distinguished by cypselas' size, shape, and apex. Unexpectedly, these genera are morphologically keyed out into separate groups due to the spines and sexuality of the capitula (Perveen &

Qaiser, 2021). Despite that, Panero (2007b) corroborated the *Parthenium* and *Xanthium* into subtribe Ambrosiinae (tribe Heliantheae) due to the absence of ray corolla and adjacent disc florets to ray florets in his molecular studies. Similarly, Robinson (1981) also placed these genera into subtribe Ambrosiinae because of obcompressed cypselas without striations. In our results, both genera are closely linked and in agreement with phylogenetic studies based on molecular data. Similarly, cypselas morphology was fairly valuable in the delimitation of species for instance, the shape and size of cypselas play an important role in the delimitation of *X. spinosum* and *X. strumarium*. Morphologically, leaf shape and size were also useful for the taxonomic delimitation of *Xanthium* at the specific level (Perveen & Qaiser, 2021).

The present result concluded that the cypselas morphological characteristics of the studied species of Heliantheae (Asteraceae) serve as diagnostic traits for the identification of taxa at the generic and specific levels and resolve many taxonomic problems. The data obtained from cypselas morphology (with cluster analysis) strongly correlated with gross morphology and was quite helpful in the systematics of the tribe Heliantheae

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