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# FOLIAR CHARACTERISTICS AS AN AID FOR THE SPECIFIC DELIMITATION OF THE GENUS *CLEOME* L. (CLEOMACEAE) FROM PAKISTAN

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**Abstract**

Leaf morphology and epidermal characters (including stomata and trichomes) of 11 species of the genus *Cleome* L. from Pakistan were studied by light and scanning electron microscopy. The genus *Cleome* is characterised by the presence of amphistomatic leaves with great polymorphism in shape. Amongst the stomatal type, actinocytic and anomocytic stomata are most prevalent in the genus *Cleome*. While, anisocytic type is exclusive for *C. ariana* and paracytic type is the characteristic of *C. brachycarpa*. All the species of the genus *Cleome* have different types of indumentum with the exception of *C. oxypetala*. Among the types of indumentum glandular hairy type is most dominant within the genus. Similarly, the quantitative characters of leaves such as, leaf size (6 − 80 × 1 – 20 mm) and stomatal frequency (154 to 769 stomata/mm$^2$) are well correlated with that of the qualitative features of leaves to strengthen the delimitation of Pakistani species belonging to the genus *Cleome*.

**Key words:** *Cleome*, Leaf morphology, Stomata, Trichomes, SEM.

## Introduction

In Pakistan the genus *Cleome* L., is represented by 11 species. Most of the species in the genus *Cleome*, especially their leaves have long been used for medicinal purposes (Burkill, 1985; Waithaka & Chweya, 1991; Schippers, 2000; Mnzava & Ngwerume, 2004). Although the floral characters are much important in identification of taxa but in the absence of floral parts, vegetative characters including leaves may also play a significant role for the proper identification. The genus shows great variations in terms of foliar characters (Jafri, 1973). Leaf characteristics along with other morphological characters have long been used by various taxonomists for specific delimitation of the genus *Cleome* (Boissier, 1867; Hooker, f., 1875; Blatter, 1919; Bobrov, 1939; Hutchinson & Dalziel, 1954; Blakelock & Townsend, 1980; Thulin, 1993; Mia *et al*., 2007; Tucker & Vanderpool, 2010). But there is no detailed information on leaf ornamentation within the genus *Cleome* available with the exception of few reports such as, Aleykutty & Inamdar (1978) described trichomes and stomata of family Capparidaceae including 8 species of *Cleome* and utilized the data for taxonomic purpose. Similarly, Edeoga *et al*., (2009) described micromorphology of vegetative and floral organs of 3 species of *Cleome* but they did not delimit taxa on the basis of data obtained. Perveen *et al*., (2007) studied the stomatal types of 69 dicot species including 2 species of the genus *Cleome*. Joshi *et al*., (2015) studied stomata and Puri (1971) analysed the trichomes of single species i.e., *C. viscosa* L.

The purpose of the present study was to investigate the leaf shape, size, ornamentation (venation, stoamata and trichomes) to provide strength to the taxonomic decisions within genus *Cleome* from Pakistan.

## Materials and Methods

Leaves of 11 species of the genus *Cleome* were collected from field along with Karachi University herbarium specimens (Appendix-I) (Fig. 1) were studied for morphology, stomata and trichomes. Leaves were directly observed under stereomicroscope (Nikon XN model) and a 1 cm$^2$ part of leaf was mounted on metalic stub by double adhesive tape and gold plated in sputtering chamber for 6 minutes followed by observation under scanning electron microscope (JSM-6380 A). Terminologies used in the present study are in accordance to Metcalfe & Chalk (1950), Lawrence (1970) and Stearn (1983).

**Appendix I. List of voucher specimens.**

| S. No. | Species | Collector, number and herbarium |
|---|---|---|
| 1. | *Cleome ariana* | A. Ghafoor & S. Omer 3019 (KUH); S. Abedin 8003 (KUH); M. Qaiser & A. Ghafoor 6592 (KUH); Kamal A. Malik & S. Abedin 1473 (KUH) |
| 2. | *C. brachycarpa* | M. Qaiser & A. Ghafoor 3 (KUH); S.M.H Jafri 813 (KUH); Shamim Akhter s.n. (KUH) |
| 3. | *C.dolichostyla* | A. Ghafoor & S. Omer 1863 (KUH); A.G. Miller & J.A. Nyberg M9569 (KUH); M. Qaiser & Asad Raza 1043 (KUH) |
| 4. | *C. fimbriata* | S.I. Ali 540 (KUH) |
| 5. | *C. karachiensis* | Sana 102 (KUH) |
| 6. | *C. oxypetala* | Herbier J. Leonard 5856 (KUH); S.I. Ali 1033 (KUH) |
| 7. | *C. pakistanica* | V. P. Dutta 5 (KUH) |
| 8. | *C. rupicola* | Sultan-ul-abedin & Abrar Hussain 6801, 6943 (KUH); S. Nazimuddin, S. Abedin & Hameedullah 557 (KUH); Jafri 2689 (KUH) |
| 9. | *C.scaposa* | Kamal A. Malik, M. Qaiser, Saood Omer & Gohar Khan 2123 (KUH); Sultan-ul-abedin & M. Qaiser 9328 (KUH); A. Ghafoor & M. Qaiser 386 (KUH); A. Ghafoor & Steve A. Goodman 4938 (KUH); M. Qaiser 2563 (KUH) |
| 10. | *C. spinosa* | Jafri 4010 (KUH) |
| 11. | *C. viscosa* | M. Qaiser & A. Ghafoor 4867 (KUH); Sultan-ul-abedin 4007, 4008, 4009, 5059 (KUH) |

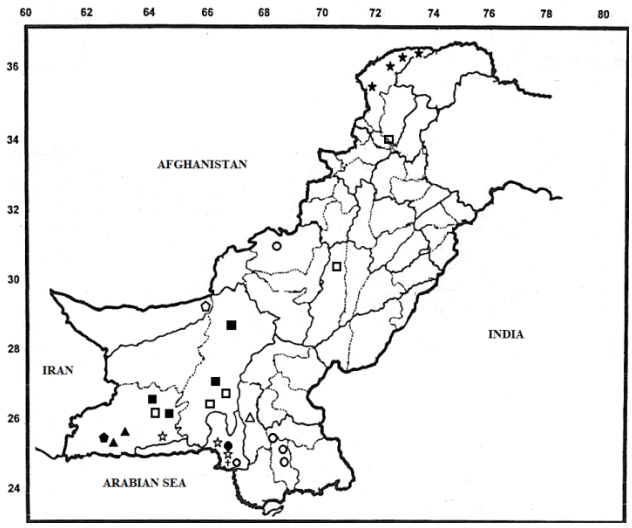


Fig. 1. Distribution pattern of studied specimens. *C. ariana* (★), *C. brachycarpa* (☆), *C. dolichostyla* (▲), *C. fimbriata* (△), *C. karachiensis* (┼), *C. oxypetala* (⬟), *C. pakistanica* (⬠), *C. rupicola* (■), *C. scaposa* (□), *C. spinosa* (●) *C. viscosa* (○).

## Observations and Results

**General leaf morphological characters of *Cleome* L.:** Leaves simple or compound (3−7 foliate); linear, lanceolate, linear-elliptic, oblong, obovate, elliptic, oblanceolate, orbicular, cordate, broadly cordate, suborbicular, suborbicular-broadly ovate, obovate-oblanceolate, elliptic-obovate, ovate-elliptic, elliptic-lanceolate, ovate, oblong-lanceolate, broad elliptic; acute, acute-acuminate, obtuse, obtuse-apiculate, obtuse-acute; base obtuse-cuneate, cuneate, truncate, cordate, attenuate; 1−5 nerved; size 6–80 × 1–20 mm with or without spiny stipules (Table 1).

**Leaf stomatal characters of *Cleome* L.:** Stomata anisocytic, paracytic, actinocytic or anomocytic, sunken or at the level of epidermal cells, 154–769/mm$^2$, aperture ovate, elliptic or oblong, aperture 5.56–21.4µm long and 1.25–11.9µm broad (Table 2) (Figs. 2 & 3).

**Leaf trichomes characters of *Cleome* L.:** Leaves usually hairy or sometimes glabrous, pubescent, scabrous or hirsute; unicellular or multicellular; glandular or eglandular, unbranched; trichomes with or without spiny projections; base broad; head pointed, capitate, peltate, globular or irregular (Table 3) (Fig. 4).

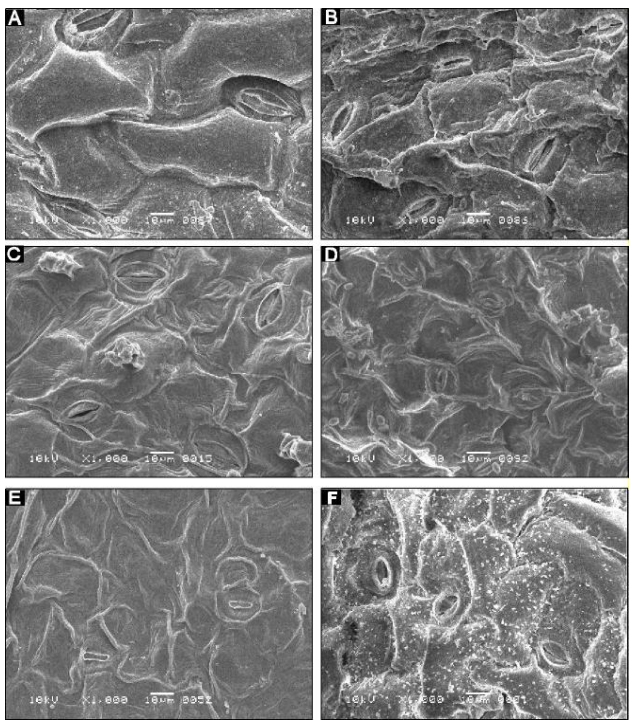


Fig. 2. Scanning electron micrographs showing leaf stomata. **A**, *C. ariana*; **B**, *C. brachycarpa*; **C**, *C. dolichostyla;* **D**, *C. fimbriata*; **E**, *C. karachiensis*; **F**, *C. oxypetala* (Scale bars: A, B, C, D, E, F = 10 µm).

### Key to species

1 + Leaves glabrous ……..…… *C. oxypetala*
- Leaves hairy ……..…….……..…… 2
2 + Leaves simple ……..…….……..… 3
- Leaves compound ……..…….……... 7
3 + Leaves hirsute, base truncate or cordate ..…..…….……..… *C. dolichostyla*
- Leaves scabrous or pubescent, base not truncate or cordate ..…..……..….. 4
4 + Trichomes with spiny projections on both surfaces of leaves ...…..…… *C. scaposa*
- Trichomes smooth on both surfaces of leaves ..……...……..… 5
5 + Stomata anomocytic ..……...……..… *C. rupicola*
- Stomata actinocytic ……...……..…… 6
6 + Leaves suborbicular-broadly ovate, scabrous, glandular, stomatal apperture oblong ...…….….. *C. fimbriata*
- Leaves elliptic, pubescent, eglandular, stomatal aperture elliptic ……...……... *C. pakistanica*
7 + Spiny stipules present, leaves oblong-lanceolate, eglandular ……..……..… *C. spinosa*
- Spiny stipules absent, leaves not oblong-lanceolate, glandular ..……...……..… 8
8 + Stomata sunken, stomatal apperture ovate, trichomes head peltate ……...……..… 9
- Stomata at the level of epidermis, stomatal aperture oblong, trichomes head capitate ……...……..… 10
9 + Stomata anisocytic ……...…..… *C. ariana*
- Stomata paracytic ……..… *C. brachycarpa*
10 + Stomata actinocytic ..……..…... *C. karachiensis*
- Stomata anomocytic ..……..… *C. viscosa*

**Table 1. Leaf morphological characters of *Cleome*.**

| S. No. | Species | Leaves simple or compound | Leaf shape | Apex | Base | Veins | Size (mm) | Stipules |
|---|---|---|---|---|---|---|---|---|
| 1. | *Cleome ariana* | 3 foliate | Linear, lanceolate, linear–elliptic, oblong, obovate | Obtuse | Obtuse−cuneate | 1-nerved | 9–20 × 1–6 | - |
| 2. | *C. brachycarpa* | 3-5 foliate | Elliptic, obovate, oblanceolate, oblong or orbicular | Acute, obtuse | Cuneate | 1-nerved | 6–20 × 2–8 | - |
| 3. | *C. dolichostyla* | Simple | Cordate, broadly cordate, suborbicular | Acute-acuminate, obtuse or obtuse-apiculate | Truncate or cordate | 5-nerved | 14–23 × 12–20 | - |
| 4. | *C. fimbriata* | Simple | Suborbicular-broadly ovate | Obtuse-acute | Obtuse−cuneate | 3–5 nerved | 7–18 × 6–11 | - |
| 5. | *C. karachiensis* | 3–5 foliate | Obovate-oblanceolate | Acute | Cuneate | 1-nerved | 6–16 × 3–9 | - |
| 6. | *C. oxypetala* | Simple | Elliptic or elliptic-obovate | Acute | Obtuse or base cuneate | 1–3 nerved | 16–25 × 4–10 | - |
| 7. | *C. pakistanica* | Simple | Elliptic | Acute or obtuse | Cuneate | 1-nerved | 8 × 2 | - |
| 8. | *C. rupicola* | Simple | Ovate-elliptic, elliptic-lanceolate | Acute | Cuneate | 1–3nerved | 13–19 × 3–11 | - |
| 9. | *C. scaposa* | Simple | Elliptic, ovate or orbicular | Acute or obtuse | Obtuse or cuneate | 1-nerved | 10–21 × 8–18 | - |
| 10. | *C. spinosa* | 5–7 foliate | Oblong-lanceolate | Acute | Cuneate | 1-nerved | 11–80 × 3–20 | Two spiny stipules are present |
| 11. | *C. viscosa* | 3–5 foliate | Elliptic, broad elliptic, oblanceolate, lanceolate or obovate | Acute or obtuse | Cuneate, attenuate | 1-nerved | 14–32 × 3–17 | - |

**Table 2. Leaf stomatal characters of *Cleome*.**

| S. No. | Species | Type of stomata | Position | Stomatal frequency (No. of Stomata/mm$^2$) | Aperture | | |
|---|---|---|---|---|---|---|---|
| | | | | | Shape | Length (µm) | Width (µm) |
| 1. | *Cleome ariana* | Anisocytic | Sunken | 231 | Ovate | 5.58(7.64)8.21 ± 0.8340 | 2.87(3.01)4.01 ± 0.4608 |
| 2. | *C. brachycarpa* | Paractyic | Sunken | 462 | Ovate | 18.6(20)21.4 ± 1.0672 | 7.54(8.43)9.21 ± 0.6355 |
| 3. | *C. dolichostyla* | Actinocytic | At the level of epidermal cells | 462 | Elliptic | 16.9(18.35)19.8 ± 1.2812 | 8.38(10.14)11.9 ± 1.3761 |
| 4. | *C. fimbriara* | Actinocytic | At the level of epidermal cells | 539 | Oblong | 6.58(8.27)9.89 ± 1.1537 | 3.1(4.15)5.3 ± 0.8843 |
| 5. | *C. karachiensis* | Actinocytic | At the level of epidermal cells | 154 | Oblong | 5.97(6.86)7.18 ± 0.5858 | 1.25(1.42)1.59 ± 0.1237 |
| 6. | *C. oxypetala* | Actinocytic | Sunken | 308 | Elliptic | 11.8(13.2)15.5 ± 1.3046 | 4.61(5.87)6.85 ± 0.7730 |
| 7. | *C. pakistanica* | Actinocytic | Sunken | 385 | Elliptic | 5.56(6.73)7.84 ± 0.9233 | 3(3.88)4.96 ± 0.6862 |
| 8. | *C. rupicola* | Anomocytic | At the level of epidermal cells | 385 | Elliptic | 12.3(14.95)17.6 2.1637 | 7.76(8.05)8.34 ± 0.2367 |
| 9. | *C. scaposa* | Anomocytic | At the level of epidermal cells | 692 | Elliptic | 13(13.6)14.6 ± 0.5211 | 3.81(4.47)5.14 ± 0.6223 |
| 10. | *C. spinosa* | Anomocytic | At the level of epidermal cells | 769 | Elliptic | 9.9(10.6)11.3 ± 0.6955 | 2.94(4.4)5.87 ± 1.1453 |
| 11. | *C. viscosa* | Anomocytic | At the level of epidermal cells | 769 | Oblong | 7.5(8.1)9 ± 0.5429 | 1.95(2.34)3.48 ± 0.6205 |

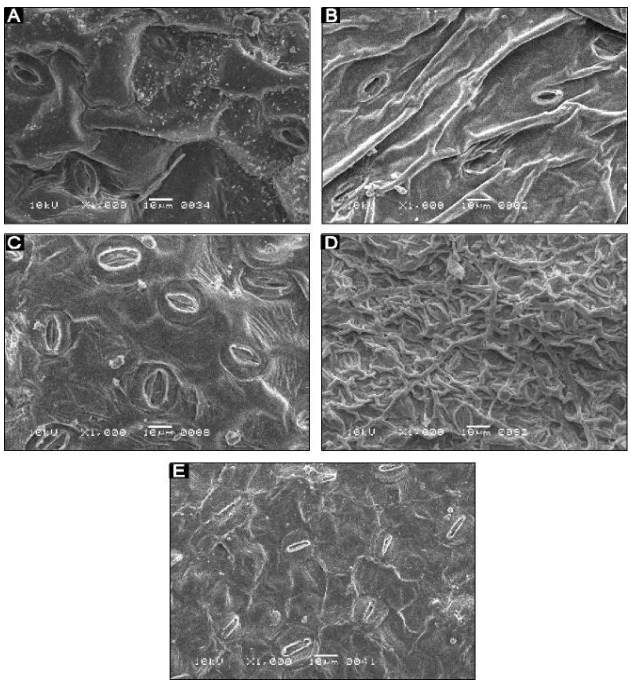


Fig. 3. Scanning electron micrographs showing leaf stomata. **A**, *C. pakistanica*; **B**, *C. rupicola*; **C**, *C. scaposa;* **D**, *C.spinosa*; **E**, *C. viscosa* (Scale bar: A, B, C, D, E = 10 µm).

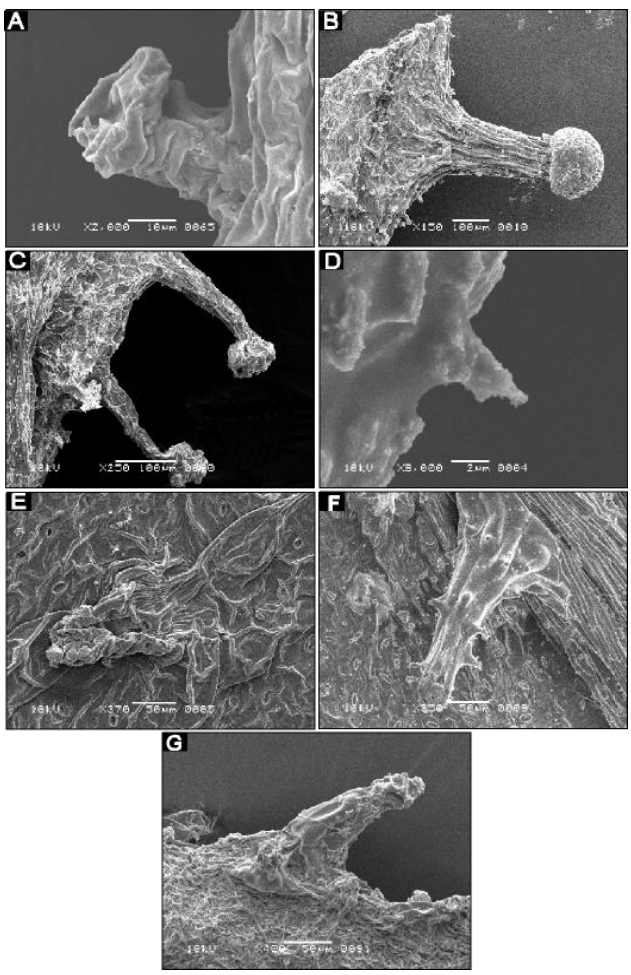


Fig. 4. Scanning electron micrographs showing trichomes. **A**, *C. ariana*; **B**, *C. dolichostyla*; **C**, *C. fimbriata*; **D**, *C. pakistanica*; **E**, *C. rupicola*; **F**, *C. scaposa*; **G**, *C. spinosa* (Scale bar: A = 10 µm; B, C = 100 µm; D = 2 µm; E, F, G, = 50 µm ).

**Table 3. Leaf trichome characters of the genus *Cleome*.**

| S. No. | Species | Indumentum | Type of indumentum | Location | Head | Trichome type | Trichome base |
|---|---|---|---|---|---|---|---|
| 1. | *Cleome ariana* | Present | Pubescent, glandular | Mostly towards the midrib and margin | Peltate | Multicellular, smooth, unbranched | Broad |
| 2. | *C. brachycarpa* | Present | Pubescent, glandular | All over leaf | Peltate | Multicellular, smooth, unbranched | Broad |
| 3. | *C. dolichostyla* | Present | Hirsute, glandular | All over leaf | Globular | Multicellular, smooth, unbranched | Broad |
| 4. | *C. fimbriata* | Present | Scabrous, glandular | All over leaf | Irregular | Multicellular, smooth, unbranched | Slightly broad |
| 5. | *C. karachiensis* | Present | Pubescent, glandular | All over leaf | Capitate | Multicellular, smooth, unbranched | Broad |
| 6. | *C. oxypetala* | Absent | - | - | - | - | - |
| 7. | *C. pakistanica* | Present | Pubescent, eglandular | Scanty, few trichomes present all over leaf | Pointed | Unicellular, smooth, unbranched | Broad |
| 8. | *C. rupicola* | Present | Pubescent, glandular | All over leaf | Peltate | Multicellular, smooth, unbranched | Broad |
| 9. | *C. scaposa* | Present | Scabrous, eglandular | All over leaf | pointed | Spiny projections on surface, unbranched | Swollen, broad |
| 10. | *C. spinosa* | Present | Pubescent, eglandular | All over leaf | Irregular | Multicellular, smooth, unbranched | Broad |
| 11. | *C. viscosa* | Present | Pubescent, glandular | All over leaf | Capitate | Multicellular, smooth, unbranched | Broad |

## Discussion

Genus *Cleome* is characterized by polymorphic leaves. The leaves of genus *Cleome* are amphistomatic (Gill *et al.*, 1982) with actinocytic, anomocytic, anisocytic and paracytic stomata. The first two types are found to be dominant while, anisocytic and paracytic stomata are specific to *C. ariana* and *C. brachycarpa* respectively. However, the previous report of MetCalf & Chalk (1950) was in contrast to the present finding where they observed only anomocytic stomata throughout Capparidaceae. Similarly, a good correlation of small sized leaves and sunken stomata was found in *C. araiana*, *C. brachycarpa*, *C. oxypetala* and *C. pakistanica*. While, remaining species with comparatively larger leaves had stomata at the level of epidermal cells. On the other hand, all the species of the genus *Cleome* were hairy except *C. oxypetala* (Jafri, 1973). Similarly, on the basis of simple or compound leaves all the species can be bifurcated (Bobrov, 1939; Jafri, 1973 & 1977; Raghavan, 1993; Chaudhary, 1998; Mathur, 2002).

Mostly, the species of *Cleome* have distinct combinition of leaf characteristics as well as few leaf features are exclusively present such as, *C. karachiensis* stood separate from all other species by the presence of obovate-oblanceolate leaves, smallest stomatal apperture width (1.25−1.59 µm) and lowest frequency of stomata (154 stomata per $mm^2$). Similarly, *C. spinosa* remained distinct by the presence of spiny stipules (Jacobs, 1960; Jafri, 1973; Iltis, 1991), 5−7 foliate, oblong-lanceolate and largest leaves (11−80 × 3–20) within the genus. While, *C. rupicola* stood out by the, presence of ovate-elliptic (Hedge & Lamond, 1970), elliptic-lanceolate leaves. *C. dolichostyla* is the only species within the genus having hirsute trichomes, 5-nerved leaves (Hedge & Lamond, 1970) and truncate, cordate leaf base and *C. fimbriata* is characterized by its suborbicular-broadly ovate, 3-5 nerved leaves having 539 stomata per $mm^2$. Furthermore, *C. scaposa* remained distinct by having spiny projections on trichomes surface. Likewise, *C. pakistanica* is the only species within the genus *Cleome* having smooth, unicellular trichomes and distinctly small leaves. Thus, from the above discussion it is evident that the data obtained from leaf morphology can be significantly used for making strong taxonomic decisions for the specific delimitation within genus *Cleome* from Pakistan.

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