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# *Culex sarpangensis*, a new species of the subgenus *Culiciomyia* Theobald of the genus *Culex* Linnaeus (Diptera: Culicidae) from Bhutan

PRADYA SOMBOON[1], JASSADA SAINGAMSOOK[1,*], RINZIN NAMGAY[2] & RALPH E HARBACH[3]
[1]*Center of Insect Vector Study, Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*
*pradya.somboon@cmu.ac.th;* *https://orcid.org/0000-0002-0760-4363*
*jassada.s@cmu.ac.th;* *https://orcid.org/0000-0001-8429-6889*
[2]*Vector-Borne Disease Control Programme, Ministry of Health, Gelephu 31101, Bhutan*
*rinzin69@yahoo.com;* *https://orcid.org/0000-0003-1697-7230*
[3]*Department of Science, Natural History Museum, Cromwell Road, London SW7 5BD, UK*
*r.harbach@nhm.ac.uk;* *https://orcid.org/0000-0003-1384-6972*
**Corresponding author:* *jassada.s@cmu.ac.th*

## Abstract

A new species of the subgenus *Culiciomyia* Theobald of the genus *Culex* Linnaeus is described from larvae found in a stream margin in Umling Gewog, Sarpang District of Bhutan. Based on morphology, the larvae are sufficiently distinct from the larvae of other species of the subgenus to be described and formally named here as *Culex sarpangensis* Somboon, Namgay & Harbach, **sp. nov.** The species is a member of the Fragilis Group. The fourth-instar larva is described, illustrated and compared with similar larvae of other species.

**Key words:** Mosquito, Taxonomy

## Introduction

The subgenus *Culiciomyia* Theobald, 1907 of the genus *Culex* Linnaeus, 1758 includes 58 species (Cornel *et al*. 2020; Wilkerson *et al*. 2021), which are divided into 5 groups based primarily on morphological characters of adults and larvae, and also zoogeography: The Dispectus Group (Bram 1969) (3 Oriental species), the Fragilis Group (Edwards 1932) (22 Oriental and Australasian species), the Nebulosus Group (Edwards 1932) (21 Afrotropical species), the Shebbearei Group (unnamed "group or complex" of Sirivanakarn 1977) (9 Oriental species) and the Tricuspis Group (Harrison 1987) (3 Oriental species). Species of *Culiciomyia* are recorded from tropical and subtropical areas, but a few Oriental species extend into the eastern Palaearctic Region (China, Japan and Korea). Some Oriental species are widely distributed, but many others have limited distributions, e.g. *Cx. lampangensis* Sirivanakarn, 1973 is known only from Lampang Province of Thailand (Wilkerson *et al*. 2021).

Adults of *Culiciomyia* are separated from the adults of other subgenera of *Culex* by combinations of the following salient characters: 1) Thoracic pleura usually without scale-patches on the mesokatepisternum and mesepimeron, 2) acrostichal setae rarely present, 3) vein 1A ending beyond the apex of crossvein mcu and 4) thoracic pleura pale or with a distinct dark to light brown area extending from the postpronotum to the upper mesokatepisternum and/or on the lower mesokatepisternum. Larvae differ from the larvae of the other subgenera by combinations of the following characters: 1) Seta 3-P distinctly shorter and weaker than setae 1,2-P, 2) seta 1-S not on lateral line of siphon, 3) pecten spines short, each with denticles of similar size, 4) setae 5,6-C long, seta 5-C about as long as seta 6-C, 5) dorsomentum of head with more than 25 narrow teeth, 6) ventral brush (seta 4-X) usually with 4 pairs of setae, occasionally 5 or 6, and 7) seta 2-X single (Bram 1967; Sirivanakarn 1971). Adults of many species of *Culiciomyia* are often difficult to separate based on external morphology, but most of them can be readily identified by features of the male genitalia and the larval stage. Larvae can be identified by combinations of characters, e.g. presence/absence of spicules on the integument of the thorax and abdomen, the shape or number of branches of setae

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1,5,6,7-C, 1,2,3-P and 6,7-I,II, the shape and number of comb scales and pecten spines, development of seta 1,4-X, the size of the saddle, the form of the anal papillae, the shape of the siphon and the development of seta 1-S.

The anopheline fauna of Bhutan is well documented because of the significance of species of the genus as vectors of malarial parasites: 38 species are known to occur in the country (Namgay *et al*. 2018; Somboon *et al*. 2022a, 2022b, 2024). In recent years, several new species of *Anopheles* have been described from the country: *Anopheles* (*Anopheles*) *bhutanensis* Somboon, Namgay & Harbach, 2022 and *An.* (*Ano.*) *monticola* Somboon, Namgay & Harbach, 2022 of the Baileyi Complex (both described in Somboon *et al*. 2022a); *An.* (*Ano.*) *druki* Somboon, Namgay & Harbach, 2022, *An. himalayensis* Somboon, Namgay & Harbach, 2022 and *An.* (*Ano.*) *thimphuensis* Somboon, Namgay & Harbach, 2022 of the Lindesayi Complex (all three described in Somboon *et al*. 2022b); and *An.* (*Ano.*) *sarpangensis* Somboon, Namgay & Harbach, 2024 of the Barbirostris Complex (described in Somboon *et al*. 2024). In contrast, the culicine fauna of Bhutan has received much less attention and is not well known. However, a number of new species have been described from the country since 2020: *Aedes* (*Hulecoeteomyia*) *bhutanensis* Somboon & Harbach, 2020 (described in Somboon *et al*. 2020); *Culex* (*Cule*x) *bhutanensis* Somboon, Namgay & Harbach, 2021 (described in Somboon *et al*. 2021b) and *Cx.* (*Cux.*) *longitubus* Somboon, Namgay & Harbach, 2021 (described in Somboon *et al*. 2021a) of the Mimeticus Subgroup; and *Uranotaenia* (*Pseudoficalbia*) *bhutanensis* Somboon, Namgay & Harbach, 2022 (described in Somboon *et al*. 2022c). So far, at least four species of *Culiciomyia* have been found in Bhutan: *Cx. nigropunctatus* Edwards, 1926, *Cx. pallidothorax* Theobald, 1905, *Cx. sasai* Kano, Nitahara & Awaya, 1954 and a species near *Cx. hainanensis* Chen, 1977 (P. Somboon, unpublished).

During recent surveys in Bhutan, we collected *Culiciomyia* larvae from a stream margin in Sarpang District. Attempts to identify the specimens using all available keys for the identification of Oriental and Australasian species were unsuccessful because many morphological characters of the Bhutanese larvae are clearly distinct from those of previously described species. However, the larval stage of seven Oriental species of the subgenus is unknown, including *Cx. bahri* (Edwards, 1914) from Sri Lanka; *Cx. delfinadoae* Sirivanakarn, 1973 from the Philippines; *Cx. fuscicinctus* King & Hoogstraal, 1946 from Western New Guinea, Indonesia; *Cx. ramakrishnii* Wattal & Kalra, 1965 from Dehra Dun, India; *Cx. ruthae* Peters, 1958 from Papua New Guinea; *Cx. tricuspis* Edwards, 1930 from Indonesia; and *Cx. yaoi* Tung, 1955 from Kiangsi, China (Wilkerson *et al.* 2021). These species, except *Cx. ramakrishnii* and *Cx. yaoi*, appear to be restricted to insular areas located more than 2,000 km from Bhutan and have not been found in countries neighboring Bhutan (China, India and Nepal). *Culex ramakrishnii* was described from a highland area in the western Himalayas, about 1,300 km from Sarpang District of Bhutan, and *Cx. yaoi* was described from an area in eastern China, about 2,700 km from Bhutan. The two species were described a long time ago and appear to have limited distributions as they are known only from their type localities. Therefore, based on geographical and environmental conditions, and morphological distinctions from known species, we are confident that the Bhutanese larvae belong to a heretofore unrecognized species, which is described below as a new species of the subgenus *Culiciomyia*.

## Material and methods

A survey for mosquito larvae was carried out in a rural area of Umling Gewog in Sarpang District of Bhutan on 31 October 2017. Larvae were collected by dipping along a stream margin and transferred to the laboratory of the Vector-borne Disease Control Programme in nearby Gelephu town. Due to limited time and facilities, all larvae were killed in hot water (about 60°C) for a few seconds and preserved in 80% ethanol. The larvae were mounted on microscope slides in Hoyer's medium (Neo-shigaral, Shiga Konchu Fukyusha, Tokyo, Japan), the slides were dried in an oven at 42–45°C for a few days, and the larvae were subsequently examined under a bright-field microscope equipped with a calibrated eyepiece micrometer. The morphological keys of King & Hoogstraal (1946), Bram (1967), Sirivanakarn (1973, 1977), Sirivanakarn & Kurihara (1973), Chen (1977, 1989), Tanaka *et al.* (1979), Toma *et al.* (1984), Harrison (1987), Tsukamoto (1989), Yang *et al.* (1993), Dong *et al.* (2003) and Rattanarithikul *et al.* (2005) were used for identification and species comparisons. The morphological terminology of Harbach & Knight (1980) is used in the description below. This article and the nomenclatural information it contains are registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:8968391F-7A97-48B5-8694-E59163B0572A. The LSID number of the new species is: urn:lsid:zoobank.org:act:6B4471BF-B404-4BD5-9D25-75FF56DD4195.

### *Culex* (*Culiciomyia*) *sarpangensis* Somboon, Namgay & Harbach, sp. nov.

Fourth-instar larva (based on three specimens) (Fig. 1a–d). Small, pale yellowish. Chaetotaxy in Table 1. *Head* (Fig. 1a): Width 0.74–0.78 mm (mean 0.75 mm), length 0.59–0.62 mm (mean 0.60 mm), more yellow than thorax, abdomen and siphon, integument smooth. Dorsomentum somewhat triangular, with 27–31 minute teeth (Fig. 1b). Seta 1-C fine, slightly curved mesally; setae 5,6-C long, with 3 aciculate branches; seta 7-C long, with 4 or 5 aciculate branches; setae 8–10-C usually with 2 simple branches; setae 11,12-C with 3–6 simple branches; seta 13-C with 3–5(3) aciculate branches. *Antenna*: 0.5 length of head width, slightly bent laterad, with many long spicules on dorsal surface, relatively strong spicules from base to seta 1-A, few small or minute spicules beyond seta 1-A, ventral surface with sparse minute spicules. Seta 1-A with 15–20 long aciculate branches, inserted about 0.66 from base; seta 2,3-A subapical. *Thorax*: Integument without covering of spicules. Setae 1,2,3-P single, long, aciculate, seta 3-P distinctly weaker and about 0.5 length of setae 1,2-P; setae 4,5,6,8,12-P single, strong, aciculate; seta 7-P double, aciculate; setae 9,10-P single, weak, simple; seta 14-P single; seta 1-M with 2–4(3) simple branches, much longer than seta 2-M but shorter than seta 3-M; seta 3-M single, simple; seta 4-M single or double; setae 5,6,7,10,12-M single, strong, aciculate; seta 9-M with 3 strong aciculate branches; setae 1,2,3,4,6-T simple, weak, with 1 or more branches; setae 7,9-T with 3–5 strong aciculate branches; seta 10-T single, strong, aciculate; seta 13-T with 4 simple branches. *Abdomen*: Seta 1-I,II,III,VI usually with 1 or 2 weak branches, 1-IV,V,VII single, noticeably long, strong, aciculate, 1-VIII with 3 aciculate branches; seta 6-I,II with 2 strong aciculate branches, 6-III,IV,V,VI single, strong, aciculate; seta 7-I single, strong, aciculate, 7-II,III,IV,V weak, with 3–6 simple branches, 7-VI,VII single, weak, simple; seta 13-I single, small, 13-II small, with 6–10(6) branches, 13-III,IV,V,VII with 2 or 3 branches, 13-VI weak, with 9–14(12) branches. Comb with 34–52 scales, scales rounded and evenly spiculate apically, lateral spicules present on some scales. Saddle incomplete, lightly pigmented, surface with rows of minute spicules and posterior margin with strong and weaker spicules. Seta 1-X single, simple, as long as saddle, inserted near edge of saddle; seta 2,3-X single, simple; seta 4-X (ventral brush) with 4 pairs of setae (setae 4a–4d), 3 on grid, 4a inserted anterior to grid, small, single, about 0.33 length of 4b, 4b single or double, about 0.5 length of 4c, 4c about 0.75 length of 4d, both setae with 2, 3 or 4 branches, branches of 4c and 4d arise a noticeable distance from the base of the stem. Anal papillae lanceolate, 2.5–3.0 length of saddle. *Siphon*: Length 1.37–1.47 mm (mean 1.42 mm), same colour as abdomen, moderately long, index 6.08–6.90, wide at base, distal 0.5 evenly narrower; acus present; pecten with 7–9(9) spines, each with fine proximal denticles; seta 1-S weak, inserted distal to pecten, shorter than width of siphon at point of attachment, comprised of 3 pairs of ventrolateral setae, 2 proximal pairs single, distal pair widely spaced from proximal pairs, single or double; setae 2,6,7,8,9-S single, weak.

**TABLE 1.** Number of branches (mode) for larval setae of *Cx. sarpangensis* (3 specimens).

| Seta | Head | Thorax | | | Abdomen | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | C | P | M | T | I | II | III | IV | V | VI | VII | VIII | X |
| 0 | 1 | 7–12 | - | - | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 1 | 1 | 1 | 2–4(3) | 2,3(2) | 2 | 1–3(2) | 2 | 1 | 1 | 1–3(1) | 1 | 3 | 1 |
| 2 | - | 1 | 3,4(3) | 1,2(1) | 1,2(1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 3 | 1 | 1 | 1 | 2,3(3) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 4,5(4) | 1 |
| 4 | 1 | 1 | 1,2(2) | 3–5(3) | 4–7(5) | 3 | 1–3(2) | 1,2 | 3–5(3) | 1,2(2) | 1 | 1 | 4 pairs |
| 5 | 3 | 1 | 1 | 1 | 2–4(3) | 2,3(2) | 2 | 1,2(2) | 1,2(2) | 1,2(1) | 1,2(1) | 2 | - |
| 6 | 3 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 6–10(9) | - | - |
| 7 | 4,5 | 2 | 1 | 4,5(4) | 1 | 3 | 2,4 | 4–6(4) | 4,5(4) | 1 | 1 | - | - |
| 8 | 1,2(2) | 1 | 3 | 5–8(5) | - | 1 | 1,2(1) | 1,2(2) | 2,3(2) | 2–4(2) | 2–5(3) | - | - |
| 9 | 2,3(2) | 1 | 3 | 3,4(3) | 1–3(2) | 1 | 1 | 1,2(1) | 1 | 1 | 1,2(1) | - | - |
| 10 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1,2(1) | - | - |
| 11 | 3–5(4) | 2–5(3,4) | 3,4 | 1,2(2) | 2,3(2) | 2 | 1,2(1) | 2,3(2) | 2,3(2) | 1–3(2) | 1,2 | - | - |
| 12 | 3–6(4,5) | 1 | 1 | 1 | 1,2 | 1 | 2,3(2) | 1–3(1) | 1 | 1 | 1,2(1) | - | - |
| 13 | 3–5(3) | – | 7–12(11) | 4 | 1 | 6–10(6) | 2,3 | 3 | 2,3(3) | 9–14(12) | 2 | - | - |
| 14 | 1 | 1 | 5–12(10) | - | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 15 | 2,3 | - | - | - | - | - | - | - | - | - | - | - | - |

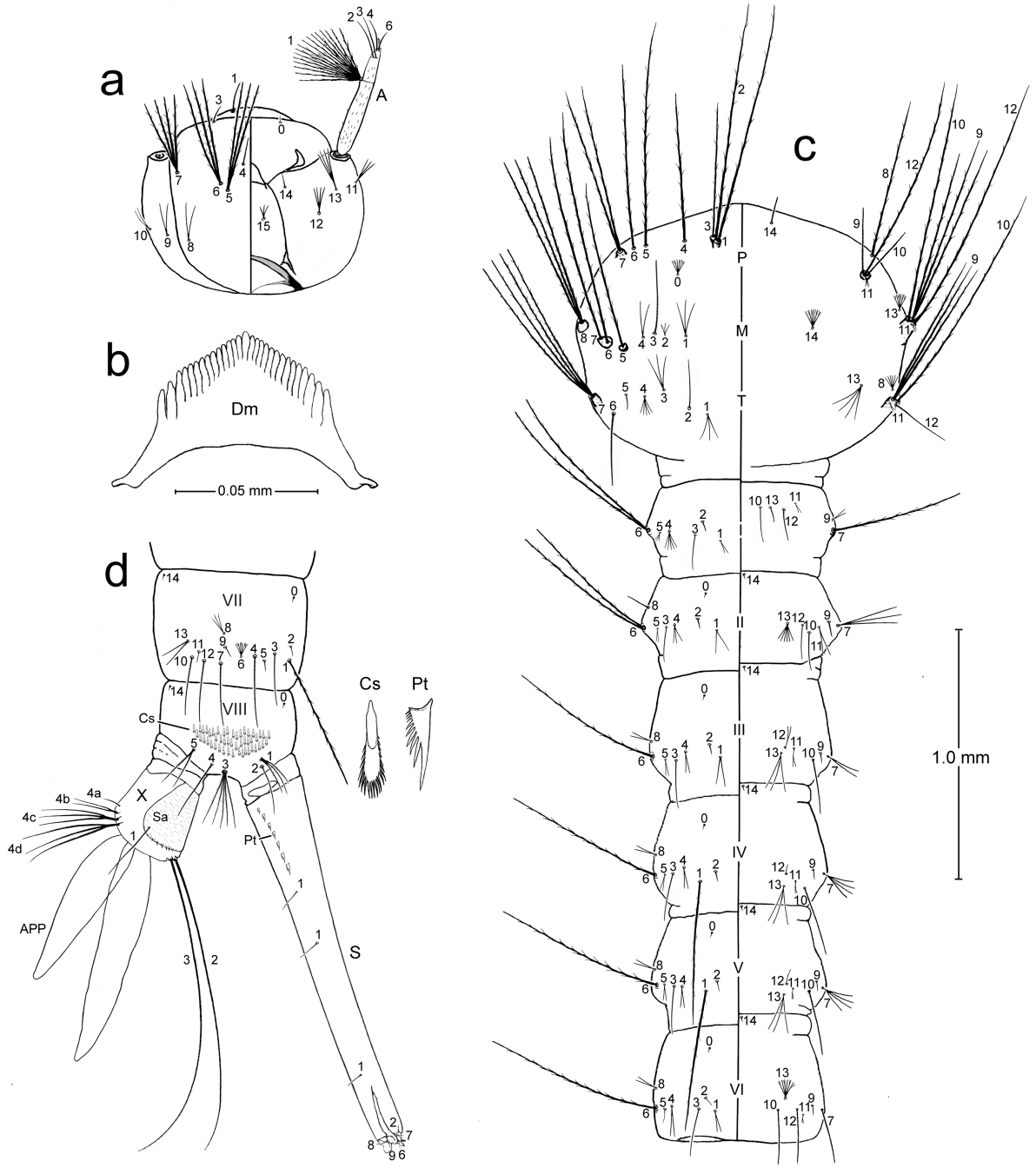


**FIGURE 1.** a–d, Fourth-instar larva of *Cx. sarpangensis*. (a) Head, dorsal (left) and ventral (right) aspects of left side, (b) dorsomentum, (c) thorax and abdominal segments I–VI, dorsal (left) and ventral (right) aspects of left side, and (d) abdominal segments VII, VIII, X and siphon, left side. A, antenna; APP, anal papillae; C, cranium; Dm, dorsomentum; P, prothorax; Pt, pecten; M, mesothorax; S, siphon; Sa, saddle; T, metathorax; I–VIII,X, abdominal segments I–VIII and X; 1–15, setal numbers for specified areas, e.g. seta 3-P and 4a.

Etymology. The specific name, *sarpangensis*, is an adjective formed by adding *-ensis* (L. masculine and feminine suffix denoting place, locality, country) to the name of the Sarpang District, the type locality in Bhutan, to produce a Latinized name meaning "from Sarpang".

Type series. Three larvae. *Holotype* (CxSp2): Bhutan, Sarpang District, Umling Gewog (26.852722 N, 90.533333 E, elevation 220 m), found in stream margin, 31 October 2017, collector Pradya Somboon. *Paratypes*:

Same data as holotype. The holotype and paratype (CxSp1), mounted on separate microscope slides, are deposited in the collection of the Entomology Section, Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

Bionomics. Larvae of *Cx. sarpangensis* were collected from the margin of a slow running stream, about 30 cm deep and partially shaded. Associated species included *Cx. nigropunctatus* and *Anopheles peditaeniatus* (Leicester, 1908). The adults and pupa are unknown.

Distribution. This species is only known at present from Umling Gewog, Sarpang District, Bhutan.

## Discussion

Larvae of *Culiciomyia* species occurring in the Oriental and Australian Regions can be divided into two groups, based on setae 1,2,3-P. In species of the first group, which includes *Cx. sarpangensis*, *Cx. fragilis* Ludlow, 1903, *Cx. nigropunctatus*, *Cx. scanloni* Bram, 1967 and *Cx. spathifurca* (Edwards, 1915), these setae are single (Rattanarithikul *et al.* 2005), whereas in the second group (with known larvae) at least one of these setae is branched, as referenced above. Larvae of *Cx. sarpangensis* can be separated from those of the other four species of the first group by several features, summarized in Table 2. Females of *Cx. fragilis* and *Cx. spathifurca* are inseparable; these two species slightly differ from *Cx. scanloni* in having darker abdominal terga and erect scales not restricted to the occiput (Rattanarithikul *et al*. 2005). *Culex nigropunctatus* adults are unique in having the thoracic pleura with a black spot on the upper mesepimeron. Unfortunately, no adults of *Cx. sarpangensis* were available for comparison. *Culex sarpangensis* can be classified in the Fragilis Group as its larva shares several characters with the larvae of *Cx. fragilis, Cx. nigropunctatus*, *Cx. scanloni* and *Cx. spathifurca*, which are all members of this group.

**TABLE 2.** Salient anatomical characters that distinguish the fourth-instar larvae of *Cx. sarpangensis*, *Cx. fragilis*, *Cx. nigropunctatus*, *Cx. scanloni* and *Cx. spathifurca*.

| Character | *Cx. sarpangensis* | *Cx. fragilis* | *Cx. nigropunctatus* | *Cx. scanloni* | *Cx. spathifurca* |
|---|---|---|---|---|---|
| Seta 5-C | 3 branches | 5–8 branches | 3 or 4 branches | 3 branches | 3 branches |
| Seta 6-C | 3 branches | 5–8 branches | 3 or 4 branches | 2 branches | 3 branches |
| Seta 4-P | single | 2 branches | 2 branches | 2 branches | 2 branches |
| Seta 8-P | single | 2 branches | 2 branches | 2 branches | 2 branches |
| Seta 14-P | single | 2 branches | single | single | single |
| seta 6-I,II | 2 branches | 3 branches | 3 branches | 3 branches | 4 branches |
| Seta 6-III | single | 2 branches | single | single | single |
| Seta 7-I | single | 2 branches | 2 branches | 2 branches | 2 branches |
| Seta 4-X | 4 pairs | >4 pairs | 4 pairs | 4 pairs | 4 pairs |
| Saddle | incomplete | complete | complete | complete | complete |
| Siphon index | 6 | 5–6 | 9–11 | 9.5–10.5 | 5–6 |
| Seta 1-S | 3 pairs, usually single | 3 pairs, 2 or 3 branches | 3 pairs, single or 2 branches | 3 pairs, single or 2 branches | 3 pairs, single |

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