Pollen morphology of *Petopentia* and *Tacazzea* (Periploaceae)

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The pollen morphology of five species of *Tacazzea* Decne. and the monotypic genus *Petopentia* Bullock has been studied. All the species of the two genera are characterized by pollen grains arranged in tetrads. The arrangement of the grains may be rhomboidal, tetragonal, linear, T-shaped, tetrahedral or decussate. Linear and T-shaped tetrads were only observed in *Petopentia*. The 4–6 pores are restricted to the junction area of adjacent grains. Exine is smooth and covered with a thin electron-dense layer. Endexine is present above the well-developed intine. The internal walls are perforated. The pollen grains of tetrads are connected by wall bridges (cross-wall cohesion). *Petopentia* and *Tacazzea* can be distinguished on pollen morphological characteristics.

Die stuifmealmorfolgie van vyf spesies van *Tacazzea* Decne. en die monotipiese genus *Petopentia* Bullock is bestudeer. Al die spesies van die twee genera word gekenmerk deur stuifmealkorrels wat in tetrades gerangskik is. Die rangskikking van die stuifmealkorrels kan romboidaal, tetraagonaal, lineêr, T-vormig, tetraëdre of kruisgewys wees. Lineêre en T-vormige tetrades is net in *Petopentia* waargeneem. Vier tot ses poriee kom voor en hulle is beperk tot die aansluitingsgebied tussen aangrensende stuifmealkorrels. Die eksien is glad en met ’n dun elektronligte laag bedek. Endeksien is bokant die goed-ontwikkelde intien aanwesig. Die interne wande is geperforeer en die stuifmealkorrels van tetrades verbind deur wandbrûe (dwarswand-kohesie). *Petopentia* en *Tacazzea* kan op grond van stuifmealmorphologiese kenmerke onderskei word.

**Keywords:** Periploaceae, *Petopentia*, pollen morphology, *Tacazzea*

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Introduction

The family Periploaceae was formerly a subfamily (Periplocoideae) of the Asclepiadaceae, but raised to family status by Schlechter (1924), Bullock (1957), Hutchinson (1959) and Dyer (1975). Of the 50 genera included in the Periploaceae, 18 occur in Africa. Among these, *Raphionacme* Harv. is the largest genus with 35 species endemic to Africa (Verhoeven & Venter 1988). *Raphionacme* is also the only herbaceous genus, although a number of its species are climbers. Most of the other genera are lianous and a small number are shrubs.

The African taxa of the Periploaceae are under taxonomic revision by the present authors. This paper on the pollen of *Petopentia* and *Tacazzea* constitutes part of a comprehensive palynological investigation of the African taxa of the Periploaceae to determine whether morphology of the pollen could supplement taxonomic evidence in a systematic study of the genera, and to present results which might be considered in a future clarification of the affinities of the genera in the family. The use of a wider range of characters frequently results in a clearer and more accurate understanding of generic limits.

In their study on the pollinaria of the Asclepiadaceae Schill & Jäkel (1978) investigated 408 species in 114 genera by light, scanning and transmission electron microscopy, and showed that the morphology of the pollinaria of the Periplocoideae differs from that of the Cynanchoideae. The Periplocoideae have pollen grains united in tetrads, whilst the Cynanchoideae have pollinia consisting of single pollen grains. The translators of the Periplocoideae are spoon-shaped or cornet-shaped with a viscidium attached. The pollinia of the Cynanchoideae consist of a corpuscle and two caudicles. Schill & Jäkel (1978) investigated nine genera of the Periplocoideae. Data on tetrad size and number of pores for *T. venosa* and *P. natalensis* are given by Schill & Jäkel (1978).

Taxonomic aspects and distribution of *Petopentia* and *Tacazzea*

*Petopentia* is a monotypic genus. *P. natalensis* (Schltr.) Bullock is found in subtropical Natal (South Africa) only. It is a liane associated with rocky habitat in places of moderate precipitation and climate. The flowers are much larger than those of *Tacazzea* and are yellowish-green in colour.

*Tacazzea* comprises five species (Table 1). *T. apiculata* Oliv. is a large liane of tropical moist forests and is most widely distributed all over tropical and subtropical Africa wherever swampy conditions occur. Its ability to maintain itself in a variety of situations is reflected in its great and often confusing morphological variability. *T. conferta* N.E. Br. is also a large liane found on the fringes of mountain forests, especially the bamboo forests at 2 000–3 000 m above sea level. Both *T. apiculata* and *T. conferta* bear conspicuous sprays of reddish to greenish-yellow multi-flowered cymose inflorescences. *T. tomentosa* Bruce is rather rare and inhabits savanna in Uganda, Sudan and Ethiopia. It is a climber over low trees and bears small cymes of creamy to
Table 1 Species and voucher specimens from which pollen was studied

| Taxon                        | Collector          | Locality            | Herbarium |
|------------------------------|--------------------|---------------------|-----------|
| *P. natalensis* (Schltr.) Bullock | A. Abbott 1241, 4 Aug. 1983 | Port Shepstone, RSA | NH        |
|                              | C. Ward 2664, 18 July 1955 | Hlabisa, RSA        | PRE       |
|                              | H. Venter 9003, 28 Aug. 1984 | Port Edward, RSA    | BLFU      |
|                              | R. Strey 6032, 14 Sep. 1965 | Pietermaritzburg, RSA | PRE    |
| *T. apiculata* Oliv.          | Versteegh & den Outer 503, 14 July 1969 | Korhogo, Ivory Coast | WAG |
|                              | K. Tinley 547, 17 Nov. 1959 | Inguaumva, RSA      | PRE |
|                              | E. Buitendag 362, 1 Dec. 1969 | Nelspruit, RSA      | PRE       |
|                              | E. Moll 4281, 4 Nov. 1969 | Ndumu Game Reserve, RSA | PRE |
| *T. conferta* N.E. Br.        | A. Stolz 1163, 17 Nov. 1911 | Kyimbila, Malawi    | WAG |
|                              | Bequaert 5927, 27 Sep. 1914 | Mukule, Zaire       | BR        |
|                              | de Wilde & Gilbert 304, 16 Feb. 1971 | Wondo, Ethiopia     | WAG |
|                              | R. Fichi-Sermolli 2193, 23 Mar. 1937 | Lago Tana, Ethiopia | MO    |
| *T. rosmarinifolia* Decne.    | J. Gossweiler 2310, 13 Nov. 1905 | Huila, Angola       | COI      |
|                              | H. Baum 245, 6 Oct. 1899 | Kubango, Angola     | G         |
|                              | E. Mendes 1678, 10 Feb. 1956 | Huila, Angola       | LISC     |
|                              | H. Jacques-Felix 493, 27 Aug. 1902 | Angola             | P       |
| *T. tomentosa* Bruce          | J. Gillet 15070, 1 Feb. 1953 | Tula, Ethiopia      | K         |
|                              | J. Wilson 844, Feb. 1960 | Loyoro, Uganda      | K         |
| *T. venosa* Decne.            | Dillon et Petit 39 | Ethiopia            | P         |
|                              | M. Schimper 1843, 1855 | Tacaze, Ethiopia    | P         |
|                              | M. St. Ange, 1850 | Nubie, Ethiopia     | P         |
|                              | M. Schimper 636, 8 Nov. 1839 | Djeladjeranne, Ethiopia |       |

Materials and Methods

Pollen was taken from either herbarium specimens or from material collected in natural habitats. Pollen sources are indicated in Table 1. For both light microscopy (LM) and scanning electron microscopy (SEM) study, pollen was acetolysed according to the method of Erdtman (1960) and prepared and investigated as described by Verhoeven & Venter (1988). Data on the measurements of length and width of rhomboidal tetrads were subjected to analysis of variance and significant differences were identified with the aid of Tukey's test (Steel & Torrie 1980).

For transmission electron microscopy (TEM) fresh material was used. Only *T. apiculata* and *P. natalensis* were examined by TEM. Pollen carriers and pollen grains (embedded in agar after osmium tetroxide fixation) were fixed in 3% phosphate-buffered glutaraldehyde (0.1 mol dm⁻³ phosphate buffer, pH 7.0), post-fixed in 1% osmium tetroxide, dehydrated in ethyl alcohol and embedded in Spurr's low viscosity resin. Sections were cut with a diamond knife, stained with uranyl acetate, followed by lead citrate, and examined with a Philips 300 electron microscope at 60 kV.

Pollen morphological descriptions

The terminology of Faegri & Iversen (1964) was used in the description of the pollen grains.

Petopentia

Pollen grains are united in uniplanar tetrads arranged either rhomboidally (Figures 1 & 5), linearly (Figures 2 & 6), or T-shaped (Figures 3 & 7). A great number of pollen grains are also arranged with three grains in a row and the fourth one slightly underneath (Figures 4 & 8). It looks like a transition form between linear and T-shaped on one hand and rhomboidal on the other. The size of
Figures 1-4 Scanning electron micrographs of tetrads of Petopentia natalensis. 1. Rhomboidal tetrad (Venter 9003). 2. Linear tetrad (Venter 9003). 3. T-shaped tetrad (Venter 9003). 4. Transition form of tetrad (Strey 6032). Scale = 10 μm in Figures 1, 2 & 4 and 1 μm in Figure 3.

the tetrads varies from 58-90 × 25-51 μm (rhomboidal) to 67-110 × 18-34 μm (linear) (Table 2). Individual grains of the tetrads have 4-6 pores, although the middle grains of the linear and T-shaped tetrads may have 8 pores. The pores are round, semi-circular or irregular and are usually opposite to each other and restricted to the junction area of adjacent grains (Figure 1). Single pores not associated with the junction area of adjacent grains are often found at the distal end of terminal grains of tetrads (Figure 4), or in middle grains of linear and T-shaped tetrads (Figures 2 & 3). Pores are sometimes covered with a thin layer of exine material (Figure 1).

Tacazzea
Pollen grains are united in uniplanar tetrads with the grains arranged tetragonally (Figure 9) or rhomboidally (Figures 10, 11, 14 & 15) or as multiplanar tetrads with the grains arranged tetrahedrally (Figures 12 & 16) or decussately (Figure 13). The most common arrangements are rhomboidal and tetrahedral. Size of tetrads varies from 36-44 × 26-45 μm (tetragonal) to 36-68 × 23-48 μm (rhomboidal) (Table 2). Individual grains of the tetrads have 4-6 pores. Pores are round, semi-circular or irregular and are usually opposite to each other and restricted to the junction area of adjacent grains (Figures 9-12). Pores are sometimes covered with a thin layer of exine material (Figure 9).

Exine structure of Petopentia and Tacazzea
The exine structure is the same in both genera. The exine is smooth and covered with a thin electron-dense layer (Figure 18). Ektexine and endexine have the same electron density but endexine has an irregular appearance because of channels which occur throughout the endexine (Figure 19). In Petopentia the ektexine is slightly thinner than the endexine but in Tacazzea the endexine is very thin in comparison to the ektexine (Figures 18 & 19). The intine is well developed with often a two-layered structure because of the loose fibrillar structure of the outer layer (Figure 18). The internal walls are perforated (Figure 17) and have the same structure as the exterior wall. The common wall between adjacent grains has wall bridges comprising intine and endexine (Figures 20 & 21). Starch grains are absent from the cytoplasm.

Discussion
The pollen morphology of the family Periplocaceae has rarely been investigated. Verhoeven & Venter (1988) reported on the pollen morphology of 35 Raphionacme species while Lebrun et al. (1984) had investigated pollen of seven Raphionacme species in their identification of Raphionacme bingeri (A. Chev.) Lebrun & Stark. Their identification of Brachystelma bingeri A.
Figures 5-8 Light microscope micrographs of tetrads of *Petopentia natalensis*. 5. Rhomboidal tetrad (*Strey 6032*). 6. Linear tetrad (*Abbott 1241*). 7. T-shaped tetrad (*Strey 6032*). 8. Transition form of tetrad (*Strey 6032*). Bar = 20 μm.

### Table 2 Diameter of tetrads (μm)

| Taxon      | Linear          | Rhomboidal       |
|------------|-----------------|------------------|
| *P. natalensis* |                 |                  |
| 1          | 81(76-87)±3,9×   | 67(58-78)±4,7×   |
|            | 24(21-27)±3,2    | 36(31-40)±3,5    |
| 2          | 71(67-76)±3,1×   | 68(58-80)±6,5×   |
|            | 22(18-24)±2,1    | 34(25-51)±6,5    |
| 3          | 87(68-110)±11,1  | 72(63-81)±6,9×   |
|            | 25(23-28)±2      | 38(32-45)±4,4    |
| 4          | 91(83-101)±5     | 82(68-90)±5,8×   |
|            | 28(25-34)±2,8    | 37(31-46)±4,9    |
| Average    | 83×25            | 71×36            |

| Taxon      | Tetragonal       | Rhomboidal       |
|------------|------------------|------------------|
|           | 2 45×34          | 54(47-63)±4,7×   |
|            | 35(32-40)±2,8    |                  |
| *T. rosmarinifolia* | 3          | 63(58-68)±3,2×   |
|            | 42(36-42)±3      |                  |
|           | 4 40×36          | 51(45-59)±3,1×   |
|            | 35(31-40)±3,1    |                  |
| Average    | 43×35            | 55×39            |

| Taxon      | Tetragonal       | Rhomboidal       |
|------------|------------------|------------------|
| *T. apiculata* | 1              | 45(43-45)±0,8×   |
|            | 40(39-42)±1      |                  |
| 2          | 36×36            | 42(36-45)±3,6×   |
|            | 39(36-41)±2,2    |                  |
| 3          | 43(39-44)±2,5×   | 45(41-46)±1,3×   |
|            | 40(36-45)±3,7    | 44(40-46)±1,9    |
| 4          | 40×36            | 42(39-45)±1,9×   |
|            | 39(36-45)±2,6    |                  |
| Average    | 40×38            | 43×41            |

| Taxon      | Tetragonal       | Rhomboidal       |
|------------|------------------|------------------|
| *T. tomentosa* | 1              | 45(40-53)±3,7×   |
|            | 27(23-30)±1,7    |                  |
| 2          | 41×26            | 42(38-47)±2,7×   |
|            | 25(23-27)±1,6    |                  |
| Average    | 41×26            | 44×26            |

| Taxon      | Tetragonal       | Rhomboidal       |
|------------|------------------|------------------|
| *T. conferta* | 1              | 54(50-64)±4,9×   |
|            | 43(36-48)±3,6    |                  |
| Average    | 41×26            |                  |
Table 2 Continued

| Taxon   | Tetragonal | Rhomboidal |
|---------|------------|------------|
| T. venosa | 43(41–45)±3,2 | 48(44–53)±3,6× |
|         | 35(35–36)±0,7 | 37(31–45)±4,5 |
| 2       | 49(42–63)±5,2× | 51(41–56)±5,8× |
|         | 40(35–45)±2,9 | 36(27–42)±5,6 |
| 3       | 38×32       | 48×38      |
| 4       | 49(42–63)±5,2× | 51(41–56)±5,8× |
| Average | 38×32       | 48×38      |

Chev. (Asclepiadaceae) as R. bingeri (Periplocaceae) was based on the presence of tetrads and absence of pollinia. In their study on the pollinaria of the Asclepiadaceae Schill & Jäkel (1978) investigated the following representatives of the Periplocaceae: Tacazzea, Periploca L., Parquetina Baill., Cryptostegia R. Br., Hemidesmus R. Br., Ectadiopsis Benth., Raphionacme, Zygostelma Benth. and Omphalogonus Baill. Data on tetrad size (seven genera) and number of pores (four genera) are given.

Compound pollen grains occur in more than 56 families of angiosperms (Erdtman 1945; Walker & Doyle 1975; Knox & McConchie 1986). The cohesion mechanisms in mature polyads were discussed by Knox & McConchie (1986). The cohesion of compound pollen

Figures 9–13 Scanning electron micrographs of tetrads of Tacazzea species. 9. T. rosmarinifolia (Jacques-Felix 493) tetragonal tetrad. 10. T. apiculata (Versteegh & den Outer 503) rhomboidal tetrad. 11. T. conferta (Beguaert 5927) rhomboidal tetrad. 12. T. apiculata (Versteegh & den Outer 503) tetrahedral tetrad. 13. T. conferta (Stolz 1163) decussate tetrad. Scale = 1 μm.
occurs by attachment of the tectum (simple cohesion) or by connecting wall bridges (cross-wall cohesion). Cross-wall connection is known to account for cohesion in at least ten families (Periplocaceae not included) (Knox & McConchie 1986). In this cohesion mechanism wall bridges are present in the common wall between adjacent grains, and these bridges may comprise several wall layers. In *Petopentia* and *Tacazzea* the bridges comprise intine and endexine. The same wall layers were also observed in *Raphionacme* (Verhoeven & Venter 1988). The family Periplocaceae can thus be added to the ten families where cohesion occurs by wall bridges. The intine wall bridges also indicate the position of pores in the acetylated pollen grains. Guinet (1965) observed pores in the internal walls of representatives of the Burmanniaceae, Caesalpiniaeae, Hydrostachyaceae and Asclepiadaceae, and interpreted the pores as a tendency towards the disappearance of these walls. Although this type of evolutionary development, from a tetrads to a monad, is an idea which is in conflict with the usual view, in some instances monads may have secondarily evolved in this way from tetrads and in such cases solitary grains represent an advanced rather than a primitive character-state (Walker & Doyle 1975).

Pollen tetrads and polyads are common in a number of families and have been used in systematic treatments to separate genera and species (Oldfield 1959; Skvarla et al. 1975; Takahashi 1986). In the Mimosoideae extensive use of tetrads and polyads is made to separate genera (Guinet 1981a, b; Niezgoda et al. 1983). In the Periplocaceae *Raphionacme* can be distinguished from *Petopentia* and *Tacazzea* by the 10-16 pores per pollen grain against 4-6 in *Petopentia* and *Tacazzea*. Four to six pores were also recorded by Schill & Jäkel (1978) for *Parquetina* and *Periploca*. The monotypic *Petopentia natalensis* can be distinguished from *Tacazzea* by the presence of linear and T-shaped tetrad arrangements which were not observed in *Tacazzea*. The average rhomboidal tetrad size of 71 x 36 μm also distinguishes *Petopentia* from *Tacazzea* where average rhomboidal tetrad size varies from 39-63 x 25-44 μm in the different species. In *Tacazzea*, tetrad arrangement is very similar in *T. apiculata*, *T. rosmarinifolia* and *T. venosa*. The majority of grains are arranged tetrahedrally or rhomboidally with the rhomboidal tetrads rectangular to spherical in form. *T. venosa* differs significantly from *T. rosmarinifolia* and *T. apiculata* in the length of rhomboidal tetrads. The arrangement of tetrads is mainly rhomboidal in *T. conferta* and *T. tomentosa*. *T. tomentosa* differs significantly from all the other taxa by the width of the rhomboidal tetrads and from *T. conferta* by the length of the rhomboidal tetrads. *Petopentia* and *Tacazzea* are characterized by starchless pollen grains, as is the case with all the Periplocaceae. According to Baker & Baker (1979) starchy pollen is a feature of...
wind-pollinated flowering plants, whereas insect-pollinated plants have starchless pollen.

This study has revealed that Petopentia, Tacazzea and Raphionacme can be distinguished on pollen morphological characteristics: Raphionacme by the 10-16 pores as against 4-6 in Petopentia and Tacazzea, Petopentia by the presence of linear and T-shaped tetrad arrangement, and Tacazzea by the 4-6 pores per pollen grain and absence of linear and T-shaped tetrad arrangement. Studies on the other genera, which are in progress, will however show whether all the African genera of the Periplocaceae can be distinguished from each other on pollen morphological characteristics. It is hoped that these pollen data will add to the systematic information on this family and will be used for comparisons within the family.

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