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Research Article

OCCURRENCE AND DISTRIBUTION OF KUMAUN HIMALAYAN AQUATIC HYPHOMYCETES: *TETRACLADIUM*

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Six species of aquatic hyphomycetes belonging to the genus *Tetracladium* (*T. marchalianum, T. setigerum, T. nainitalense, T. apiense, T. breve,* and *T. maxilliforme*) isolated from submerged leaf litter as well as roots of various riparian plants of Nainital, Kumaun Himalaya (India) are described. Former two species are found common in occurrence while latter three species are rare in occurrence. Except *T. maxilliforme,* all the species were also recovered as root endophyte. A detail taxonomic description, occurrence and worldwide distribution of each Kumaun Himalayan species of *Tetracladium* along with a simplified key is also provided.

INTRODUCTION

Aquatic hyphomycetes (also known as freshwater conidial fungi, amphibious fungi and Ingoldian fungi) are an ecologically distinct, polyphyletic group of fungi (Bärlocher, 2015). Their taxonomy and identification have traditionally been based on the morphology and ontogeny of asexually produced spores (mitospores or conidia).Since these conidial fungi abundantly occur on dead decaying submerged leaf litter of various deciduous trees in well aerated fresh water bodies, Ingold (1942) highlighted them as aquatic hyphomycetes.

Occurrence and distribution of the aquatic hyphomycetes have been studied all over the world by several workers (Tubaki 1957; Hudson & Ingold 1960; Peterson 1962; Alasoadura 1968; Park 1974; Ingold 1975; Nawawi 1975; Webster & Descals 1979; Descals & Webster 1982; Kuthubutheen *et al*. 1992; Sridhar *et al* 1992; Marvanová 1997; Gulis *et al* 2005;). Many species of these fungi have also been discovered from various habitats of Kumaun Himalaya, India (Sati & Tiwari 1990, 1993, 1997; Sati *et al*. 2002 a b, 2009).

Tetracladium is one of the first described genus of aquatic hyphomycetes (de Wildeman, 1893) and *T. marchalianum* as type species. Later *Tetracladium maxilliforme* Rostrup (1894) and *Tetracladium setigerum* Grove (1912) were added to this genus.However *T. maxilliforme* and *T. setigerum* were initially described as *Titaea maxilliformi*s and *Tridentaria setigera* respectively (Ingold, 1942).

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Untill 1981 this genus included only three species characterized by staurosporous thalloconidia and conidia with typically four divergent branches.

After nearly four decades one more species named *T. apiense* was included by Sinclair & Eicker (1981) from the South Africa followed by *T. furcatum* Descals (1983) from the Scottish Highlands. Later Roldan (1989) raised two more species as *T. breve* and *T.palmatum* from the Spain. Recently *T. nainitalense* was described by Sati *et al* (2009) from Nainital, India as a root endophyte of riparian herb*Eupatorium adenophorum.*

Currently altogether eight valid species of *Tetracladium* are known. In the present study six species of this genus recovered from Kumaun Himalaya, India are described with their taxonomic details and worldwide distribution.

MATERIALS AND METHODS

Samples of decaying submerged leaf litter were collected in sterile polythene bags from different high altitude perennial streams of Nainital, Kumaun Himalaya, India. Stream water foam and live roots of riparian plants were also sampled. The collected samples were processed in the laboratory(Sati *et al* 2014). Leaf litter were rinsed under running tap water for 4-5 hours to remove soil particles and debris. Root samples were treated with 2% sodium hypochlorite solution for surface sterilization. Leaf litter and surface sterilized roots were then cut into small pieces (4–5 cm) and placed into sterilized Petri dishes containing 20 ml of sterile water and incubated 15±2°C. After 2–3 days the incubated leaves were periodically observed under the microscope to detect the conidia of aquatic hyphomycetes, while root segments were examined after 15-20

days to detect the conidia of root endophytic aquatic hyphomycetes. Conidia were picked aseptically and placed onto 2% malt extract agar supplemented with streptomycin to obtainaxenic culture (15±2ºC, 7 - 10 days).

Foam samples were fixed with 5% FAA to check the conidia directlyunder low and high power microscope for detection of conidia. Semi-permanent slides of these conidia have been deposited in the Kumaun University Mycological Slide (KUMS) collection of the Department of Botany, Nainital. For identification agar block containing fungal hyphae were transferred into sterilizedwater for sporulation in petridish. Taxonomic details were recorded separately for sporulated conidia and species were identified with the help of pertinent literature (Roldan *et al* 1989; Sati *et al* 2009).

RESULTS

During this study conidia of many species of Aquatic Hyphomycetes were recovered from different localities of Kumaun Himalaya, India on submerged leaf litter, foam and live roots of riparian plants. Altogether six species of *Tetracladium* (*T. apiense, T. breve, T. marchalianum, T. maxilliforme, T.nainitalense* and *T. setigerum*) were identified (Table 1). Among these two species *T. marchalianum* and *T. setigerum* were found common in occurrence, while three species namely *T . apiense, T. breve* and *T. maxilliforme* were found rare in occurrence.*T. nainitalense* which was recovered first time from Nainital showed its wide distribution but rare in abundance(Table 1).

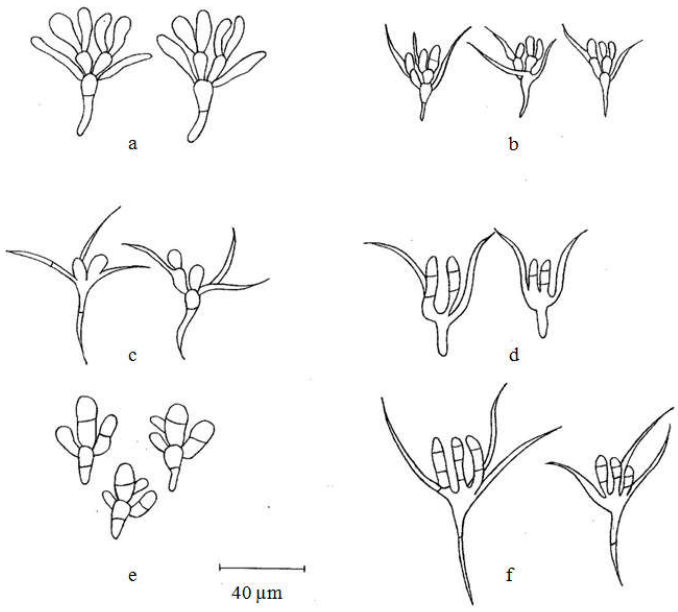


Figure 1 a- *Tetracladium apiense*; **b**-*T. breve*; **c**- *T. marchalianum*; **d**- *T. maxilliforme*; **e**- *T. nainitalense*; **f**- *T. setigerum*

Submerged aquatic fungus with branched septate mycelium. Conidiophores micro to semi acronematous, simple or loosely branched. Conidiogenous cells with denticulate scars. Conidial branches straight or very slightly curved outwards, never filiform or acicular, primary branches arising at two levels.

Table 1 Species of *Tetracladium* reported from Kumaun Himalaya, India

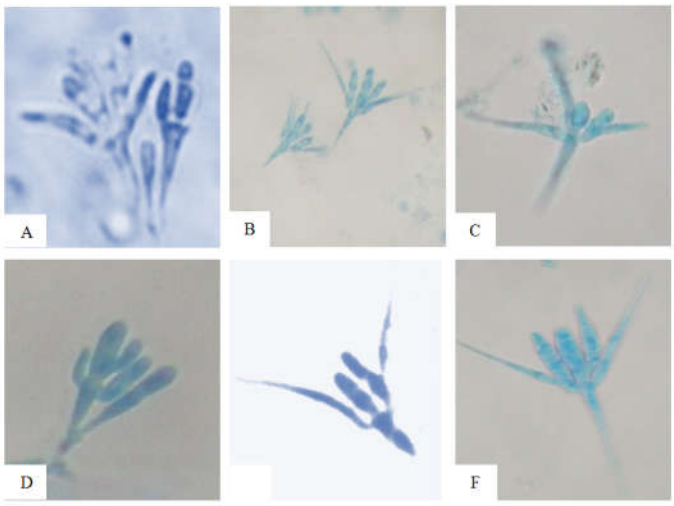
S. No.	Species	Habitat	Locality	Abundance	Worldwide geographical distribution
1.	*T. apiense**	F L (Pn, Mi) E (Ea, Vw, Ps)	JW, RT, KT	Rare	SA (Sinclair & Eicker, 1981), Sp (Roldan et al, 1989), PR (Betancourt et al 1983), In (Sati et al, 2002).
2.	*T. breve**	E (Ea, An)	GM, RT	Rare	Sp (Roldan et al, 1989), In (Arya and Sati, 2010), Br (Fiuzaa&Gusmão, 2013).
3.	*T. marchalianum**	F L (Pr, Ra, Cd, Ao, Lc, Lo, Sc, Ba, Ql, Cg) E (Ug, Ds, Gn, Eq,Cv, Qf, St, Ph)	J, VK, SV, KB, RT, JW, RG	Common	Bel (De Wildeman, 1893), Eng (Ingold, 1942), Cal (Ranzoni, 1953), J (Tubaki, 1957), NA (Peterson, 1962), Aus (Cowling & Waid, 1963), Sw (Nilson, 1964), Sp (Roldan et al, 1989), PR (Santos- Flores & Betancourt-Lopez) Eg (Ahmed, 1997), In (Mer & Khulbe, 1981).
4.	*T. maxilliforme**	L (Ql and Ao)	SV	Rare	Den (Rostrup, 1894), Eng (Ingold, 1942), Sw (Nilson, 1964), Sp (Roldan et al 1989), PR (Santos- Flores and Betancourt- Lopez), In (Sati & Joshi, 1994).
5.	*T. nainitalense***	E (Ea, Co, Lc)	GM, RG	Rare	In (Sati& Arya, 2009),NZ(Aimer & Segedin, 1985) Br(Fiuza &Gusmão, 2013).
6.	*T. setigerum*	F L (Lc, Bs, Sc, Ql, Cd, Ra) E (Gn, An, Mk, Os, Av, Or, Ug)	JW, J, KB, SV, VK	Common	Eng (Ingold, 1942), Cal (Ranzoni, 1953), J (Tubaki, 1957), Ja (Hudson & Ingold, 1960), SA (Greathead, 1961), NA (Peterson, 1962), Sw (Nilson, 1964), In (Thakur, 1977), PR (Santos- Flores & Betancourt- Lopez, 1994), Sp (Roldan et al, 1989), CR (Bills & Polishook, 1994).

Pn- *Polygonum nepalaensis*, Mi- *Mangifera indica*, Ea- *Eupatorium adenophorum*, Vw- *Valeriana wallichii*, Ps- *Pilea scripta,* An- *Alnus nepalensis*, Pr- *Pinus roxburghii*, Ro- *Rhododendron arboreum,* Cd- *Cedrus deodara*, Ao- *Acer oblungum*, Lc- *Lanatana camara,* Lo- *Lyonia ovalifolia,* Sc- *Syzigium cumini*, Ba- *Betula alanoides*, Ql- *Querecus leucotrichophora*, Cg- *Cephalotaxus griffithi*, Gn- *Geranium nepalensis*, Eq- *Equisitum* sp., Cv- *Carpessium viminea*, Qf- *Querecus floribunda,* St- *Salix tetrasperma*, Ph- *Parthanium hysterophorous*, Os- *Oxalis sp*., Co- *Colcasia* sp, B- *Barberris* sp, Mk- *Murraya koenegii*, Av- *Artemisia vulgaris*, Or- *Oenothera rosea*, Ug- Unidentified grass.
F- Foam, L- Leaf litter, E- Root Endophyte; JW – Jageshwar, RT- Ratighat, KT- Khurpatal, GM- Gufa Mahadev, RG- Ramgarh, J- Jeoli, VK- Vinayak, SV- Snow View, KB- Kilburi.
SA- South Africa, Sp- Spain, PR- Puerto Rico, In- India, Br- Brazil, J- Japan, NA- North America, NZ- New Zealand,Bel- Belgium, Eng- England, Eg- Egypt, Sw- Sweden, Cal- California, Den- Denmark, Ja- Jamaica, CR- Costa Rica.
*- New to India, **- New to Sciencefrom Kumaun Himalaya

It is interesting to note that out of eight known species of the genus *Tetracladium* six species also occurred in Kumaun Himalaya, India. Of these four species viz. *T. apiense, T. breve, T. marchalianum, T.maxilliforme* recovered from Kumaun Himalaya were new to India. Detailed taxonomic description, global distribution and key of these species of *Tetracladium* is being given here under:

Tetracladium apiense Sinclair & Eicker (Fig. 1 a, Plate 1 A) Trans. Brit. Mycol. Soc., 76: 515, 1981

Mature conidia with axis distally digitiform 25-35× 4-5 µm, 2-3 septate, base pointed. At 10-12 µm from the base three lateral branches develop, two of them subulate, 13-18×2-3 µm, 0-1 septate, third digitiform 10.5-12 ×2.5-3.5 µm, one septate bearing another subulate branch, 6.5-11 ×2-3µm aseptate, the second level of the axis is a further (rarely two) subulate element 8-10 ×3 µm aseptate. Digitiform elements appear as a pair of forked structures.



Present species isolated from the Ramgarh stream (2050 m asl) on submerged leaf including pine niddle litter in the month of March 2017. In India *T. apiense* was first recorded from Nainital, Kumaun Himalaya at Vinayak (1500 masl) on submerged leaves of *Mangifera indica* and *Polygonum nepalensis* (Sati *et al*, 2002) and also isolated from the living roots of *Eupatorium adenophorum* and *Valeriana wallichii*as root endophyte from Ratighat (1200 m asl) and Khurpatal (1600masl) (Arya & Sati, 2010).

Absence of pointed elements is the distinctive feature of this species of *Tetracladium*. Our isolate differs from those of Sinclair & Eicker (1981) and Roldan *et al* (1989) in the length of main axis and arms. It was interesting to note that present species also isolated from pine needles and living roots of riparian plants.

Geographical distribution- South Africa (Sinclair & Eicker, 1981), Puerto Rico (Betancourt *et al* 1983), Spain (Roldan *et al*, 1989) in foam and incubated leaves of *Populus nigra* and *Salix atrocinera*, India (Sati *et al*, 2002).

Tetracladium breve Roldan (Fig. 1b, Plate 1 B)
Mycol. Res., 93: 452-465, 1989

Aquatic fungus with branched and septate mycelium. Conidiophores micro to semi-macronematous and then mononematous, simple or sparsely branched, 12-32 × 1-2 µm. Conidia laterally compressed, acrogenous with a central axis and with branches sequential and pleurogenous, primary branches on consecutive cells of the axis, immediately below septum and with basal septa, diverging in one plane. Mature conidia multiseptate, axis with distal element digitiform, 13-20 × 2.5-3.5 µm, 2-3 septate, basal extension 5-7 µm long. Out of three elements two of them are opposite, one of which is digitiform, 8-10 × 1.5-2.5 µm, 1-2 septate, other acicular 12-23 × 1-2 µm, 1-2 septate; the third element is filiform and up to 20.5 µm long, the third digitiform element bears at its middle part an abaxial acicular element 5-15× 1-2 µm. on the second level and on the either side of the axis a digitiform to ellipsoid element is inserted, 5-9 × 1.5-2.5 µm, 0-1 septate.

This species isolated from the living roots of *Alnus nepalensis* at Ratighat (1200 masl) in the month of July 2017. Earlier it was isolated as root endophyte from the living roots of *Eupatorium adenophorum* from Gufa Mahadev (1850 masl), Nainital. This species was recorded as new root endophyte as well as new to Indian aquatic fungi. (Arya & Sati, 2010)

The conidia of *T. breve* are similar in structure with *T. setigerum* but smaller in size. Indian species of *T. breve* isolated only from the roots of riparian plants while in other cases it was mainly isolated from submerged leaf litter and foam samples. It shows little variation from Spanish *T. breve* as described by Roldan *et al* (1989) in the acicular branches which are larger in our isolate.

Geographical distribution- Spain (Roldan *et al*, 1989) in foam, India (Arya & Sati, 2010), Brazil (Fiuza & Gusmão, 2013) from submerged decaying leaves.

Tetracladium marchalianum de Wild. (Fig1 c, Plate 1 C)
Ann. Soc. Belge. Microsc. 17: 35, 1893

Submerged aquatic fungus with branched, septate, mycelium. Conidiophore micro to semi macronematus and simple or sparsely branched 100-115x 1-2 µm. Conidia with axis 25-35 µm long, 1-3 septate, distal cell globose or ellipsoid, 3-4 µm wide, base of the axis pointed, primary branches at one level, intermediately below the globose cell, diverging broadly in various planes, two of them acicular, 25-30 x 1.5-3 µm , 0-1 septate, the third is short, 9-13 x 3-4 µm typically, 1 septate. Apical cell globose, 3-3.5 µm wide, secondary branches acicular, 22-28 x 1.5 to 2 µm, typically 0 septate, inserted below the second globose cell.

This species was recorded from submerged leaves of *Querecus floribunda*, *Rhododendron arboretum, Pinus roxburghii* and *Cephalotaxus griffithi* from Snow View and Kilbury stream and isolated as a root endophyte from the roots of *Alnus nepalensis, Debregessia* sp, *Geranium nepalense* and Ferns in the month of November 2016 and March 2017. Earlier this species wasrecovered from Jageshwar (1775 masl), Jeoli (1150 masl), Kilbury (2000 masl), Ratighat (1200 m asl) and Vinayak (1500 masl) in submerged leaf litter and foam (Sati *et al*, 2002).

The characteristic feature of this species is presence of globose elements which easily differentiate it from other species of *Tetracladium*. In Kumaun Himalaya, India this species also isolated from various Gymnospermous leaf litter.

Geographical distribution- It is one of the most commonly occurring species reported from various part of the world showing its wide distribution. Belgium (De Wildeman, 1893), England (Ingold, 1942), California (Ranzoni, 1953), Japan (Tubaki, 1957), North America (Peterson, 1962), Australia (Cowling & Waid, 1963), Sweden (Nilson, 1964), Spain (Roldan *et al*, 1989), India (Mer & Khulbe, 1981, Sridhar & Kaveriappa, 1989, Sati & Tiwari, 1990 in submerged leaf litter, Ghate & Sridhar, 2017 as a root endophyte from the roots of *Canarium strictum*),Puerto Rico (Santos- Flores & Betancourt- Lopez- 1994) in foam and submerged leaves of *Mangifera indica* L.,Egypt (Ahmed, 1997).

Tetracladium maxilliforme (Rostrup) Ingold (Fig.1 d, Plate 1 E) Trans. Brit. Mycol. Soc., 25:339, 1942

Submerged aquaticfungus with branched, septate mycelium. Conidiophores apical or lateral, simple to loosely branched. Conidiogenous cells with truncated scars. Conidia with primary branches arising at one level. Conidia with axis distally digitiform, subclavate, 11-15×2.5-3 µm, 1-3 septate, scar truncate to rounded, without basal extension. At 4-7 µm above the scar two lateral elements develop opposite each other : one of them digitiform, 0-1 septate, the other branch

acicular, initially parallel to the axis and then curving outwards, 7-16 ×1.75-2 µm 0-1 septate. The digitiform branch bears, at its middle part, an abaxial acicular element similar to the first 16-25 × 1.5-2 µm.

Collected from snow view stream (2270 masl) on submerged leaves of *Querecus leucotrichophora* and *Acer oblongum* in the month of April 2017. It has been reported as a new record for Indian aquatic fungi (Sati and Joshi 1994).

Conidial morphology of Indian *T. maxilliforme* shows variation in size with Spanish isolate as it is smaller in size.

Geographical distribution- Denmark (Rostrup, 1894) from stems of *Trifolium pratense*,England (Ingold, 1942), Sweden (Nilson, 1964), Spain (Roldan *et al*, 1989)in foam, Puerto Rico (Santos- Flores & Betancourt- Lopez, 1994) in foam, India (Sati & Joshi, 1994).

Tetracladium nainitalense Sati and Arya (Fig 1 e, Plate 1 D) Mycologia. 101 (5), 692–695, 2009

Aquatic fungus with branched septate mycelium. Conidiophores micro- to semimacronematous, mononematous, simple or sparsely branched, up to 23 µm long. Conidiogenous cells integrated, single or grouped, acrogenous, polyblastic sympodial, scars denticulate. Detached conidia appear lobate, with all elements in a single plane, with 3–4 rounded apices. Conidial axis distally broadly digitate, proximally obconic, 17–26 × 3–4.5 µm, 2–3 septate, without basal extension; At the first level 5–11 µm above the detachment scar, two lateral elements are inserted; opposite or nearly so, broadly digitate to ellipsoid, straight orlightly curved, 6–10× 3–4.5 µm, 0–1 septate. At the second level a broadly digitiform to ellipsoid element is inserted on the axis, 3–5 × 2–4 µm, aseptate.

The typical feature of present isolate is absence of acicular branches. It was isolated as a root endophyte from roots of *Lantana camara* in the month of February2017 from Ratighat stream (1200 m asl). Earlier it was isolated as a root endophyte from the roots of *Eupatorium adenophorum* and *Colcasia* sp. (Sati *et al*, 2009).

Geographical Distribution- It appears to be a restricted species of *Tetracladium*. Although the present conidia was also reported from New Zealand as conidia of unknown species by Aimer & Segedin (1985) but isolated first from India (Sati & Arya, 2009), Brazil (Fiuza &Gusmão, 2013) in foam.

Tetracladium setigerum (Grove) Ingold (Fig. 1 f, Plate 1 F) Trans. Brit. Mycol. Soc., 25:339, 1942

Submerged aquatic fungus with branched, septate mycelium. Conidiophore usually simple, 15-35 µm long with a single terminal conidium. Conidia with primary branches arising at two levels. Detached conidia with axis distally digitiform, 30-40×3.5-4 µm, 3-6 septate. Basal extension is 7-10 µm long that form three finger like elements two of them opposite, one of which is digitiform, 15-20×3-3.5 µm, 1-3 septate, and the other acicular, 25-35×2-3 µm, 0-2 septate, the third element filiform and up to 40 µm long. The digitiform elements bears at its middle part an acicular element12-17.5 ×1-2 µm, 0-1 septate. On the second level and on either side of the axis appears another digitiform element 10-15 ×3-4 µm, 1-2 septate.

This species was recorded from Kilbury stream (2000 masl) on submerged leaves of *Querecus leucotrichphora* and also isolated as a root endophyte from the roots of *Barberries* sp in the month of March and April 2017. Earlier it was reported from submerged leaf litter and riparian roots of *Lyonia ovalifolia, Murraya koenegii*, Ferns and *Equisetum* sp. (Sati & Belwal, 2005).

The present isolate differs from Ingold (1942) on the basis of its conidial germination. The conidia of present isolate germinate mostly from the finger like projections and randomly from the divergent arms. Ingold (1942) observed germination mostly from the arms. The Indian isolate also shows variation with Roldan *et al* (1989) in the length of main axis and finger like projections.

Geographical Distribution- This species of *Tetracladium* show a wide distribution as it has been recorded from various part of the world. Grove, 1912 from leaves of *Angelica sylvestris*, England (Ingold, 1942), California (Ranzoni, 1953), Japan (Tubaki, 1957), Jamaica (Hudson & Ingold, 1960), South Africa (Greathead, 1961), North America (Peterson, 1962), Sweden (Nilson, 1964), Spain (Roldan *et al*, 1989), India (Thakur 1977, Subramanian and Bhat, 1981, Manoharachary, 1989, Mer & Sati, 1989) Costa Rica (Bills & Polishook, 1994) from leaf litter, Puerto Rico (Santos- Flores & Betancourt- Lopez, 1994).

Key to the Kumaun Himalayan species of Tetracladium

1Conidia apices not pointed …….....**2**
1' Some apices pointed ……**.3**
2 Typically six elements; appearing four elements as a pair of similar, forked structures, subulate…….....***T. apiense***
2'Typically lobate conidia having 3–4 elements with rounded apices……..……..*****T. nainitalense***
3 Two elements globose or subclavate.....***T. marchalianum***
3' Elements never so……..**4**
4Apices less than eight, two or fewer levels of primary branches….....**5**
5 Basal extension absent, conidia with never more than four apices; one level of primary branches……..……...***T. maxilliforme***
5' Basal extension present, conidia with more than four apices; two levels of primary branches ……....**6**
6With typically three digitiform elements; conidia mostly with six apices……..…….**7**
7Axis (excluding the basal extension) 17–21 3 1.5–2.5 µm ….....***T. breve***
7'Axis (excluding the basal extension) (28–) 34–42 3 4–5 µm……....……....*****T. setigerum***

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