UTILITY OF THE MACRO-MICROMORPHOLOGICAL CHARACTERISTICS USED IN CLASSIFYING THE SPECIES OF *TERMITOMYCES*

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ABSTRACTS

Delimitation of Termitomyces *species principally rely on the macro-micromorphological characteristics of the species although recently they are supplemented by molecular techniques. Utility of these characters have never been examined to authenticate their usefulness. The present paper therefore, provides a detailed study on the efficacy of the macro and micro-morphological characters used in classifying* Termitomyces *species. Macromorphologically, capsize was very useful and based on it, three groups were established. Other more useful characters include colour of the cap and pseudorrhiza presence. Annulus presence, pseudorrhiza colour, size, and morphometry were apparently unuseful. Micromorphology was less informative and ambiguously used while untetra basidiospore character was revealed for the first time in this genus. In general macromorphological characteristics provide more reliable taxonomic information to delineate most of the species in the genus than micromophology. However, for the more similar species, an analysis of more stable molecular characters is required. More distinguishing characters are yet to be revealed while ascertaining the used one and taxonomic status of* T. citriophylus *is essential.*

Key words: Characters, Macro-micromorphology, Mushrooms, Taxonomy, *Termitomyces.*

INTRODUCTION

The genus *Termitomyces* R. Heim belongs to the Superkingdom: Eukaryota, Kingdom: Fungi, Division: Basidiomycota, Class: Basidiomycetes, Subclass: Agaricomycetidae, Order: Agaricales, Family: Lyophyllaceae. It comprises fungi that live in an obligate symbiosis with termites of the subfamily Macrotermitinae. Many species of *Termitomyces* frequently form fruit bodies, which develop from the fungus comb within the nest and are all edible. Taxonomy and edibility of the species in this genus is well known and documented. From Africa they have been known to be palatable and delicious as reported by Heim (1942, 1977), Otieno (1964), Alasoadurall (1966) Pegler (1977), Pegler and Rayner (1969), Moriss (1986), Pegler and Piearce (1980), Piearce (1987), Buyck (1994), Vander Westhuisen and Eicker (1990), Botha and Eicker (1991). From Asia they have also been reported by Pegler and Vanhaecke (1994), Natarajan (1979), and Zhang (1986).

Good scientific mushroom taxonomy studies are very important in mushroom classification since poorly done taxonomy can be fatal. Like any other living organisms, mushrooms are identified through observing their identity of their characters with naked eye, using hand lens, microscopes and more recently by the use of molecular techniques. Through these methods, protocols for describing and illustrating each species are made to guide correct identification of a particular mushroom (Bougher and Katrina, 1998). In conventional mushroom taxonomy, general procedures are followed by examining and recording the characters of the basidiomes starting from the field. Macro-morphological characters easily observed in the field are recorded first, followed by further examination including microscopic characters observed on fresh or dried mushrooms. Distinguishing characteristics of the species differ between genera such that not all studied characters provide useful distinguishing traits among species of different genera. The genus *Termitomyces* embodies species of enormous macro-micromorphometry which provide useful taxonomic characters in delineating the species of the genus. This has been long taken for granted without reassessing the quality of the taxonomic characters involved in species demarcation. The present paper, therefore, provides the results of a detailed study on the utility of the macro and micro-morphological characters used in classifying *Termitomyces* species.

MATERIALS AND METHODS

Macro-micromorphology characters of twenty five species of *Termitomyces* shown in Table 1 including four species from our own collection deposited at the mycological herbarium of the "Museum National d' Histoire Naturelle Cryptogamie" at Paris (PC) and duplicates at the University of Dar es Salaam (UDSM) were included in the study. The distinguishing characteristics of the species were also noted from the literatures of Heim (1977), 17 species; Pegler and Van haecke (1994), 9 species; Van der Westhuisen and Eicker (1990), 7 species; Buyck (1994), 5 species and Härkönen *et al*. (1995, 2003), 5 species. Because none of the literature has statistical values for the measurement of basidia, cystidia and basidiospore, the mid point between the maximum and minimum values was taken to represent the measure of the features. Collection included four species from 5 localities along the coast of Tanzania namely**,** Kisarawe, Madalle, Tabata, Ubenazomozi and within the University of Dar es Salaam campus (Fig. 1).

Fresh fruit bodies were collected and examined in fresh conditions as explained in Tibuhwa *et al*. (2008). Fruiting bodies were dried by an electric drier (STOCKLI CH-8754, SWITZERLAND) for 8 hours and preserved for later microscopic studies. The microscopic features measured in micrometer included basidia, cystidia and basidiospore. The measurements were taken as mean values of 20 randomly sampled measures, measured after taking a small fragment from the hymenium of the dried specimen treated in 10% Ammonium solution contained in an aqueous solution of Congo red which stained the hyphal walls. The observations and measurements were taken on a bright field compound microscope Olympus (OM BX 50, JAPAN) with 100 x oil immersion objective and eyepiece connected to an Olympus microscopic drawing tube which helped in drawing the observed feature.

Basidiospores terms used in this study were based on Bas (1969) with basidiospore shape described in terms of length-width ratios (Q): globose = 1-1.05, subglobose = 1.06-1.15, broadly ellipsoid = 1.16-1.23, ellipsoid 1.24-1.6, oblong = 1.65-2.0 and cylindrical or sub fusi form > 2.0. Spores and cystidia were studied by scanning electron microscopy (JEOL –JSM-840, JAPAN) following the procedure of the manufacturer.

Table 1: Summary of Macro-morphological characters used as distinguishing traits of the *Termitomyces* species

Species	Iconography	Cap Colour	Cap size	Annulus	Stem-colour	Pseudorhiza
T. ***striatus* f. *striatus* (Beeli) R. Heim**	Buyck (1994), Heim (1958)	Brownish/ ochraceus brown /Pale grayish/ gray to grayish brown	medium	Absent /Present	Grayish-brown	Present
T. ***aurantiacus* (R. Heim) R. Heim**	Härkonen *etal* (1995, 2003), Heim (1977)	Ochraceous- orange	Medium	Absent	white	Present
T. ***entolomoides* R. Heim**	Heim (1977)	Bluish black	Small	Absent	bluish	Present
T. ***mammiformis* R. Heim**	Heim (1942)	Pale brown/ Whitish grey	Medium	Present and persistent	White	Present-hollow with blunt end
T. ***spiniformis* R. Heim**	Heim (1977)	Gray	Medium	Present	Off-white	Present
T. ***schimperi* (Pat.) R. Heim**	Heim (1942)	Cream-white with concentric rings of brown scales	Large	Absent, covered by thick membranous of velar remain	creamy	Present
T. ***letestui* (Pat.) R. Heim**	Buyck (1994), Härkonen *etal* (1995, 2003)	Cream to light brown	Large	Present	cream	Present
T. ***robustus* var. *robustus* (Beeli) R. Heim**	Buyck (1994), Heim (1951)	Tawny-brown/ Pale cream	Medium	Absent	Pale brown	Present
T. ***fulignosus* R. Heim**	Heim (1942)	Rustrown / Ochraceus to orange	Medium	Absent	Buffy-brown	Present
T. ***citriophyllus* R. Heim**	Heim (1942)	Dark-ochre-orange mixed with citrine yellow	Medium	not recorded	not recorded	Present

Species	Iconography	Cap Colour	Cap size	Annulus	Stem-colour	Pseudorhiza
T. ***globulus*** **R. Heim & Gooss.**	Heim & Gooss (1951)	Dull orange with pale margin	Medium	Absent	white	Present- rarely bulbous
T. ***clypeatus*** **R. Heim**	Härkonen *etal* (1995, 2003), Heim (1959)	Grayish to buff-brown paling toward the margin	Small	Absent/ Present	white	Present- near soil surface bulbous
T. ***lanatus*** **R. Heim**	Heim (1977)	Grey wooly cover	Large	Present	Gray-white	Present
T. ***albuminosa*** **(Berk.) R. Heim**	Heim (1941)	not recorded	Medium	Present, partial sometime evident	white	Present-brown and fusoid above
T. ***heimii*** **Natarajan**	Natarajan (1979)	Silky white becoming grey to brown-white-creamy	Medium	Persistent, double	white	Present - hollow
T.***microcarpus*** **f.** ***microcarpus*** **(Berk. & Broome) R. Heim**	Buyck (1994), Härkonen *etal* (1995, 2003), R. Heim (1942)	Creamy-white	Small	Absent	White	Absent
T. ***medius*** **R. Heim**	Heim (1951)	Buffy-brown	Small	Absent	white	Absent
T. ***titanicus*** **Pegler & Piearce**	Buyck (1994), Pegler & Piearce (1980)	Ash-gray with dark and brown patches	Large	Present	white	Present
T. ***sagittiformis*** **(Kalchbr. & Cooke) D.A. Reid**	Reid (1975)	Grayish-sepia pale toward the margin	Medium	Absent	Creamy-white end extending to brown pseudohiza	Present- fusoid

Species	Iconography	Cap Colour	Cap size	Annulus	Stem-colour	Pseudorhiza
T**. *umkowaani* (Cooke & Massee) D.A. Reid**	Reid (1975)	Cream pale-grey to brown buff	Large	Absent	White, swollen at the base, extending to rust brown pseudohiza	Present
T**. *reticulatus* Van der Westh. & Eicker**	Van der Westh. & Eicker (1990)	Cream-white distracted with brown scales	Small	Present	White with swollen base	Present
T**. *eurhizus* (Berk.) R. Heim**	Härkonen *etal* (1995, 2003), R. Heim (1942),	Dark-grayish brown pale toward the margin	Large	Absent	Creamy-white end nding into black pseudorhiza	Present
T**. *radicatus* Natarajan**	Natarajan (1977); Pegler & Vanhaecke (1984)	Pale orange/Grayish brown pileus and dark spiniform perforatorium	Small	Present	Off white to pale orange	Present
T**. *cylindricus* S.C. He**	S.C. He (1985)	Pale brown-ash grey	Medium	Absent	White swollen at base	Present white above, yellowish green below
T**. *singidensis* Saarim.& Härk.**	Saarim. & Härk. (1994)	Dark brown with white postulate scales	Medium	Absent	White often swollen base	Present

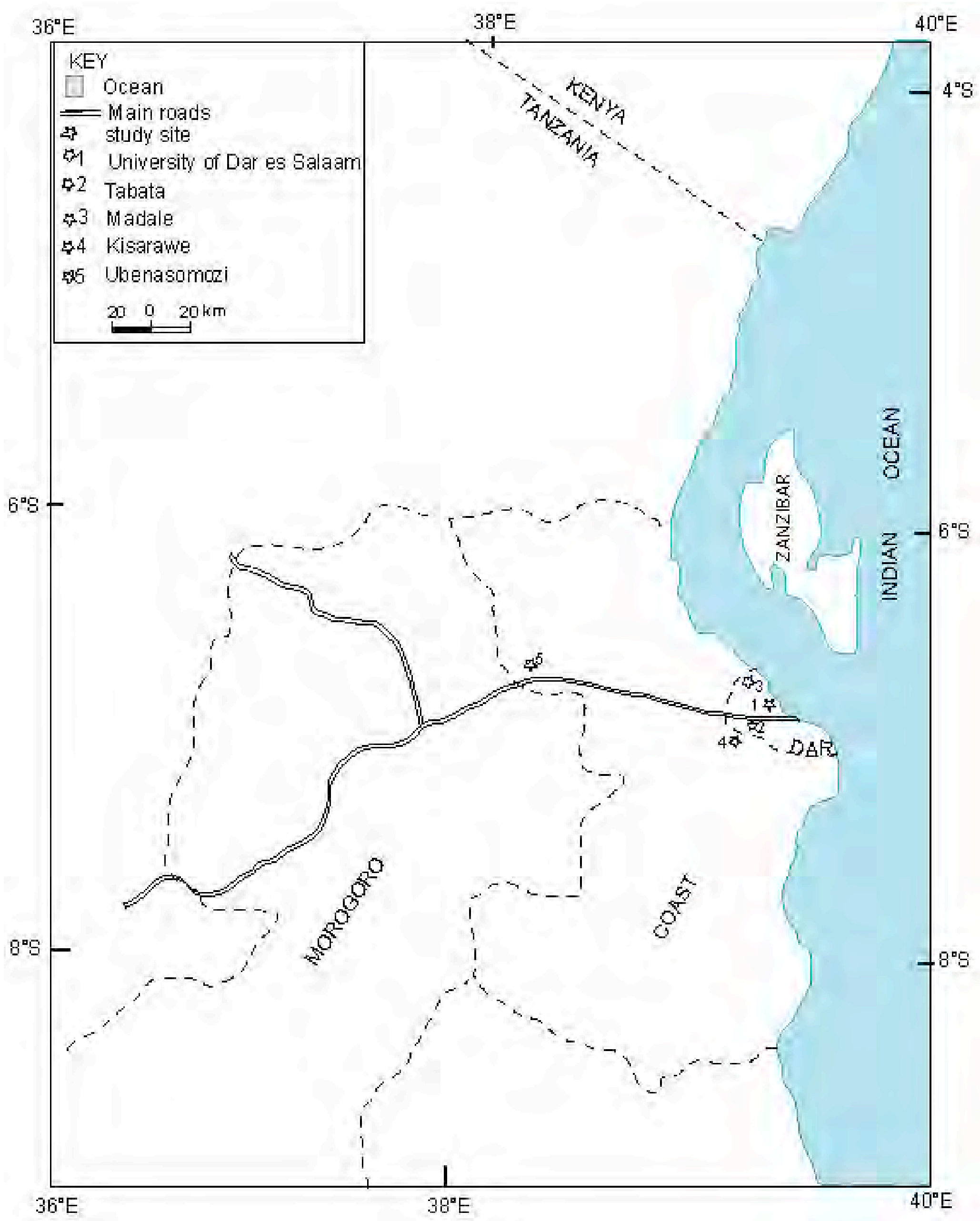

Figure 1: Map of Tanzania showing the study sites along the coastal areas.

RESULTS AND DISCUSSION

Many macro and micro-morphological characters are described in the systematics of the *Termitomyces* species. Re- assessing their utility in this study found out some of the characters which are very useful, some with little use while others provide ambiguous taxonomic information in distinguishing the species of the genus. One more distinguishing trait was revealed.

Basidioma macromorphology

Cap sizes

Caps size varies enormously between the species (Fig. 2) ranging from less than 1cm (small) to 1 meter (large). From a comparison of literature data and our own observations it follows that three broad groups of *Termitomyces* basing on cap size may be categorized as (I) the small sized cap; embodying group with cap size (Cs) $0 < Cs < 40$ mm which includes; *T. microcarpus, T. medius, T. indicus* and *T. entolomoides*; (II) the medium sized cap with (Cs); $40 < Cs < 200$ mm which include the rest of the studied species except (III) the larger sized cap; (Cs) $200 < Cs < 1000$ mm which includes *T. eurhizus, T. globulus, T. letestui, T. schimperi, T. umkowaan* and *T. titanicus*. Although often highly variable due to differences in age and environmental conditions, the size of the basidioma can be diagnostic in some instances. The groups categorized in this study (I), (II) and (III) in Fig. 2, can be very useful in narrowing the wide-ranging of comparisons in the field guide and more systematically important to the smallest size species *T. microcarpus* with cap size less than 4 cm (Heim 1977, Buyck 1994, Härkönen *et al*., 1995, 2003). In a special case such as the one of a huge mushroom *T. titanicus* with large cap size up to 1 meter (Pegler and Piearce, 1980; Piearce, 1987; Buyck, 1994), its taxon may be confirmed while in the field using this category beyond skepticism. Likewise in the case of medium sized group, the range of comparisons is narrowed, making identification work easier.

Alexopoulos (1986) mentioned "cap" as a morphological feature being environmentally influenced and Piearce (1987) reported on the effects of the architecture, depth, size and nutritional status of the parent comb and resistance of the soil to the cap but they didn't show how or explain the relationship of the effects. However, Kunugawa and Furukawa (1965) found out that temperature affects the development of fruit bodies since mushrooms have their minimal, optimal and maximal temperatures for their healthy growth while Webester (1970) noted the effect of light intensity on mushroom fruitbody morphology, often with much-elongated stipe and poor cap development depending on the light intensity. Despite all these possibilities of environmental influences, cap size remains very useful in species delimitation especially by narrowing down the range of comparison and to the two extremes the 'smallest' and 'largest taxa'.

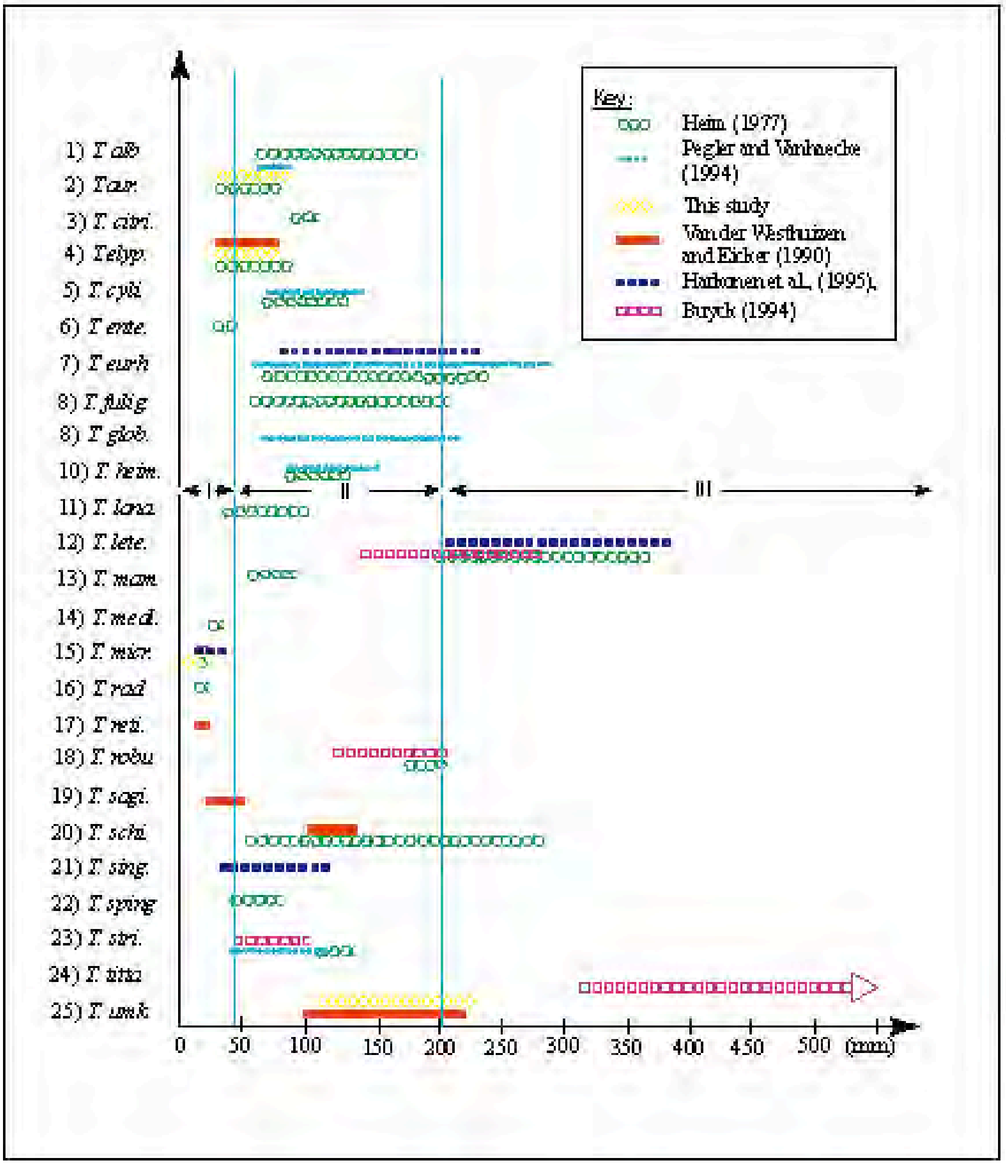

Figure 2: Three groups of *Termitomyces* species based on their cap size ranges in mm.

Basidioma colour

The colour of the cap varied from white-cream to grey, orange to brown and rarely to bluish-black between species (Table 1). Colour is much used in identification keys to narrow the broad range of comparisons for different groups of species in the genus. In *T. striatus* (Fig.3a) and *T. aurantiacus* (Fig.3b), it is the only standing delimiting character with *T. striatus* being ochraceous brown to grayish brown unlike *T. aurantiacus* which is uniformly ochraceous orange (Heim, 1977). Colours of basidioma cap, stipe and lamellar are the most useful macro-morphological characters. A major problem with this suite of characters is that pigments responsible for these colours are unknown in many groups of agarics including *Termitomyces* (Singer, 1986). Since the colour outlook between species differs markedly, assumptions of homology between the pigments in the *Termitomyces* and in other potential out-group taxa could not be made. However, much variation of colour is found within this genus from white-cream to grey, orange to brown and rarely to bluish-black between species (Table 1). Since it is very useful character in taxonomy of the species in this genus, there is thus a need for more studies to determine pigments responsible for particular suite of colour in order to make it a more stable and certain distinguishing character.

Pseudorrhiza

The pseudorrhiza length varied from none (Fig.3c) to more than one meter (Fig. 3e). This character of pseudorrhiza presence or absence was most useful in the two species which lack the pseudorrhiza (*T. microcarpus* (Fig.3c) and *T. badius*) apart from the neotropical, non-termitophilous species described by Gómez (1995). Since the colour of *T. microcarpus* is constantly known to be cream white, then further difference is put to the later species that is known to have buffy brown colour. The morphometry of pseudorrhiza also varied, some being cylindrical, others widening at certain depth then narrowing unevenly to the point of attachment to the termite nests (Figs 3d, f). Colour of the pseudorrhiza is sometimes dissimilar to that of the stipe. The aspects of colour and morphometry of the pseudorrhiza are not much used as distinguishing characters between species. For example, different species exist with similar morphometry and colour of pseudorrhiza, among them are *T. umkowaan; T. eurhizus* and *T. globulus*, all with dark coloured pseudorrhiza widening to a certain point then narrowing unevenly to the point of attachment to the termite nests (Van der Westhuisen and Eicker, 1990). The length of the pseudorrhiza is determined by the depth of the termite comb and may be absent or present in species of *Termitomyces*. Pseudorrhiza presence is very useful to the two species which lack it while its colour and morphometry are of little significance in distinguishing species of *Termitomyces*.

Annulus

Annulus varied from completely none, to microscopic squamules, to fugacious appendiculate and to very thick ring like structure encircling the stipe (Table 1). This character is very difficult to be used in the identification key because of its wide range of variation and fugacious nature. For example, in *Termitomyces clypeatus*, it is present in early stages only, thus, its presence on the basidiome depends on the developmental stage of the basidiome. In *Termitomyces striatus* f. *striatus*, it may be present or completely absent unconditionally thus provides unstable taxonomic information.

Spore prints

All the *Termitomyces* species have a pinkish spore colour in mass (Heim, 1977; Singer, 1986). The colours of the print occasionally turn pale brown with long storage (Singer, 1986). This character of pinkish spore print was very consistent in the studied species, and hence strengthening its usefulness in circumscribing the genus itself and not the species in the genus.

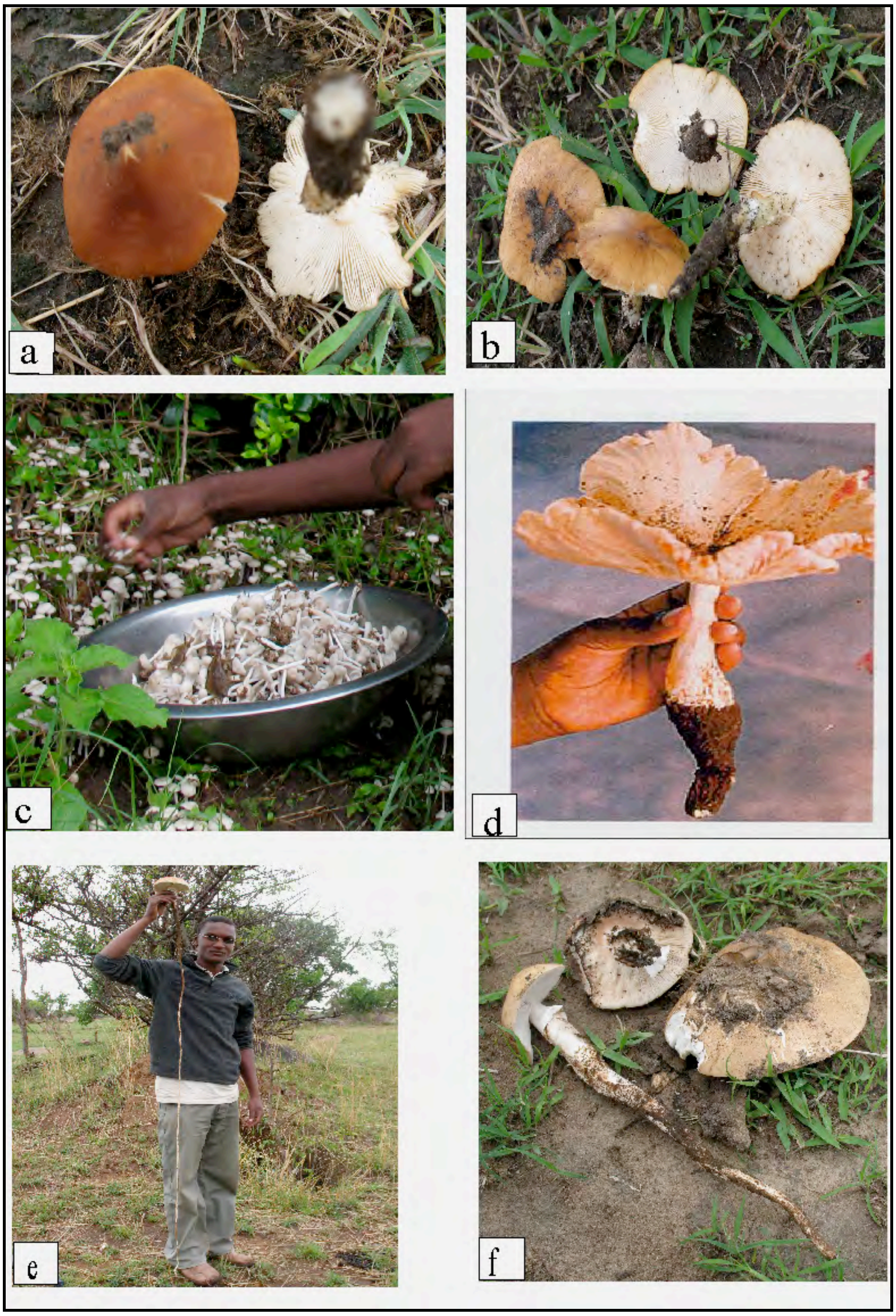

Figure 3: *Termitomyces* species basidiome (a) *T. striatus*, (b) *T. aurantiacus*, (c) *T. microcarpus*, (d) *T. umkowaan*, (e) *T. eurrhizus* pseudorhiza length 1.8 meters, (f) *T. Letestui* (*All photo by Tibuhwa DD*)

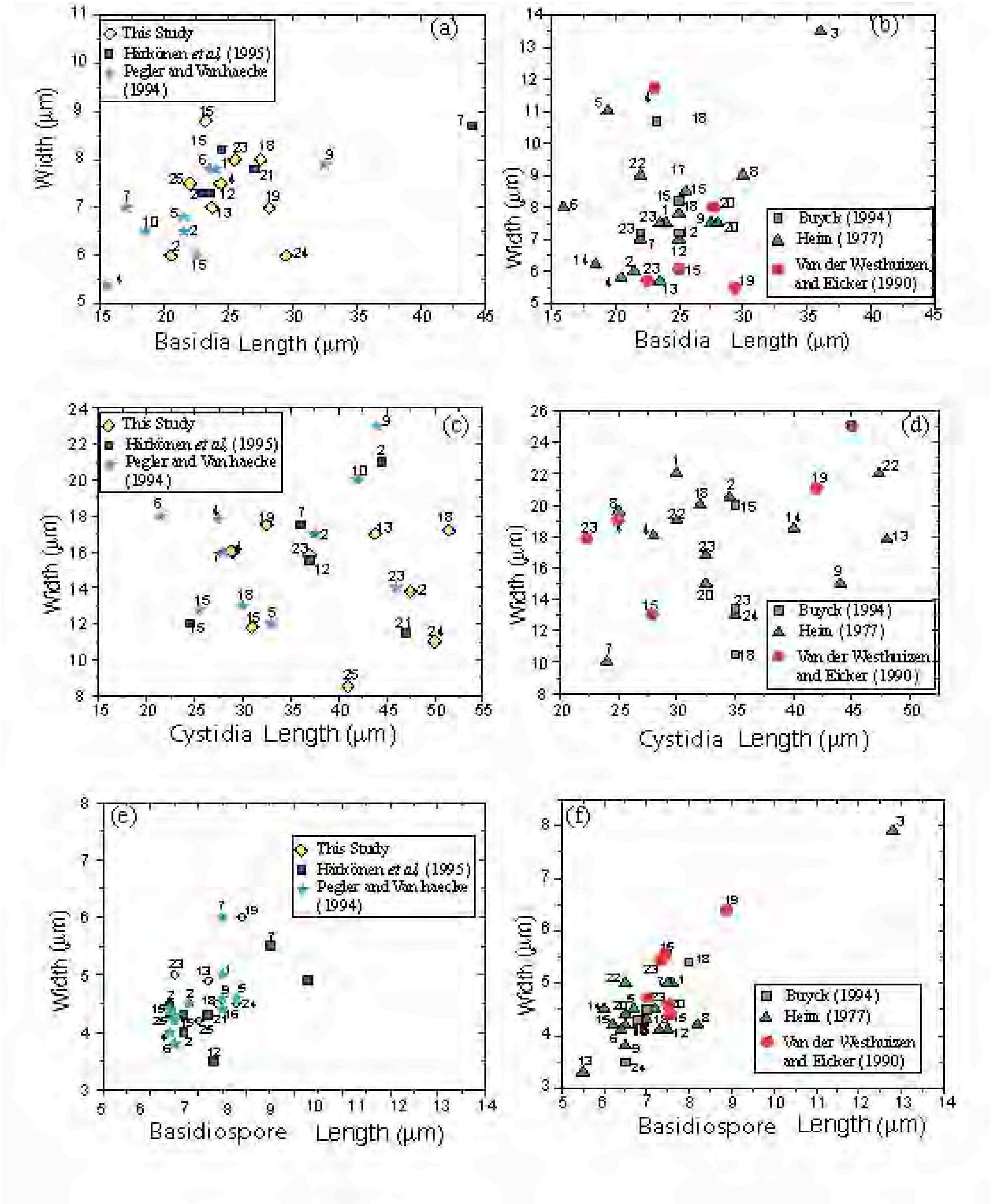

Figure 4: *Termitomyces* species microscopic measurements (length against width) in µm for (a, b) basidia, (c, d) cystidia, (e, f) basidiospore. (Numbers 1- 25 represents species given in abbreviation definition)

Definition of used abbreviations

1 T.alb.-T. albuminosa	9 *T.glob.-T. globulus*	17 *T.reti.- T. reticulatu*
2 *T.aur.- T. aurantiacus*	10 *T.heim.- T. heimii*	18 *T.rob.- T. robustus*
3 *T.citri.- T. citriophyllus*	11 *T.lan. -T. lanatus*	19 *T.sag.- T. sagittiformis*
4 *T.clyp.- T. clypeatus*	12 *T.lete.- T. letestui*	20*T.sch.- T. schimperi*
5 *T. cyli. T. cylindricus*	13 *T.ma.- T. mammiformis*	21 *T.sing.- T. singidensis*
6 *T.ente.- T. entolomoides*	14 *T.med.- T. medius*	22 *T.spin.- T. spiniformis*
7 *T.eurh.- T. eurrhizus*	15 *T.micr.-T. microcarpus*	23 *T.stri.- T. striatus*
8*T.flug.- T. fulignosus*	16 *T.rad.- T. radicatus*	24 *T.tita.-T. titanicus*
		25 *T.umk.- T. umkowaan*

Basidioma micromorphology

Basidia

The basidia measure of *Termitomyces* species was found to be 18 < L < 30 x 5< W < 9.5 for length and width, respectively, except for one species, *T. citriophyllus*, which measured 39.5 x 13.5 µm. The basidia size is not much used in the identification keys, except for more closely related species. From the results in Fig. 4 a and b, it shows that all basidia size within the genus do not differ much enough to put a clear demarcation between species except for *T. citriophyllus*, whose size diverged from the rest of the members of the genus. Unlike many other genera of agarics with varying number of sterigmata in one basidioma Singer (1986), the number of basidiospore in *Termitomyces* for a long time has been known to be four, a character used in genus circumscription (Singer, 1986). In all of the materials examined, the vast majority of the basidia observed had the same number of sterigmata (Fig. 5a), except *T. umkowaan* which showed 1-2 sterigmater (Fig. 5b) thus counterfeiting the tetrabasidospore character of the genus.

Although Van der Westhuisen and Eicker (1990) noted *T. umkowaan* to have larger basidia and basidiospore than other species of *Termitomyces*, this study found the species with largest basidia and basidiospore to be *T. citriophylus* as shown in Fig. 4 a,b and e,f, respectively. No any other basidia character appeared to have any systematic significance.

Cystidia

Cystidia size measured 24 < L < 49 x 8 < W < 28 µm for both length and width, respectively. The size of cystidia among the studied species did not differ much, even that of *T. citriophyllus* reclined within the range of other members of the genus (Fig. 4c and d). Cystidia shape was found to be useful to more similar species like *T. umkowaan* and *T. eurhizus*. Cystidia shapes stand as a difference between the two, the former being broadly clavate to pyriform (Va nder Westhuisen and Eicker, 1990) while the later being obovoid to pyriform (Pegler and Van haecke, 1994).

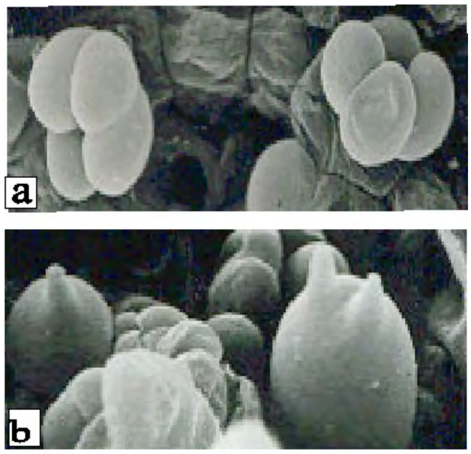

Figure 5: (a) *T. microcarpus* tetrabasidiospore (b) *T. umkowaan* untetra basidiospore shown by 1-2 young sterigmata

Basidiospores

Basidiospores of *Termitomyces* have little variation in their morphometry thus provide little taxonomic information for designating the species. Most of them are ovoid to ellipsoid shaped, the character which circumscribe the genus rather than species. Surprisingly, this study found again the divergence of the basidiospore dimension of the very species *T. citriophylus* (Fig. 4e and f). It measured 12.5 x 7.9 µm, while the rest measured 5.5 < L <8.5 x 3.5 < W < 6.5µm for length and width, respectively. Van der Westhuisen and Eicker (1990) distinguished *T. umkowaan* from *T. eurrhizus* using the basidiospore size and shape, by describing *T. eurrhizus* having somewhat large basidiospores and broadly clavate to pyriform cystidia. This study found inconsistencies in the basidiospore size such that they overlap between the two species. For example, the basidiospore of *T. eurrhizus* as reported by Pegler (1994) is 6.5-8.5 x 4 -5 µm; Härkönen *et al*. (1995) 6.8-10.0 x 4.1-6.0 µm; Heim (1977) 6.5-8.5 x 4.0-5.0µm while that of *T. umkowaan* is 6.1-11.3 x 4.3-5.6 µm as observed by Van der Westhuisen and Eicker (1990) and 6.8-8.0 x 4.3-5.6 µm observed in this study. This inconsistency and overlap of the basidiospore size limits its use in demarcating the two species. Macromorphologically, the two species are also known to resemble each other in four important aspects: (1) Both having dark-coloured pseudorrhiza, (2) Similar shape and colour of the cap, (3) Similar pileal surface with hyphae forming a hypodermal layer below the epicutis consisting of radially parallel, repent, narrow hyphae (Pegler and Rayner, 1969) and (4) Variation of the morphology of their sporocarp as noted by Van der westhuisen and Eicker (1990) for *T. umkowaan* compared to the description of *T. eurhizus* by Pegler and Rayner (1969), Pegler (1977), Pegler and Piearce (1980) and Piearce (1987). The mentioned similarities concur with the suggestion that the two species are con-specific (Van der Westhuisen and Eicker, 1990; Pegler and Van haecke, 1994). There is, therefore, a need for more scientific justification especially by using molecular systematics to ascertain the taxon status of the two species.

The untetra basidiospore was another additional distinguishing characteristic noted in *T. umkowaan*; that character was revealed for the first time in this genus (Fig. 5b). The size of basidia and basidiospores of *T.*

citriophylus diverged from the rest of the members of the genus (Fig. 4 b and f). The divergence of one species from other members of the genus suggests that it may be of a different genus (Singer, 1986; Bougher and Katrina, 1998). *Termitomyces citriophylus* R. Heim is not much well known. Its record is known from a single report by Heim 1977), who described it. Additionally, it has a yellow stipe and citrine yellow lamellae (Heim, 1977), the character not found in other members of the genus. *Termitomyces citriophyllus* has been once related to one species from India, *T. albidolaevis* Dhanch., J.C. Bhatt & S.K. Pant in terms of the size of pileus (9–10 cm) and of basidiospores (9–11 x 6.7–7.7 µm), but differs in the dark colored (ochreous gray) pileus, and much larger basidia (30–49 x 12–15 µm, compared with 17.0–27.5 x 5.0–7.5 µm in *T. albidolaevis*). This study therefore, suggests re-examination of the type material of *T. citriophyllus* to confirm its taxonomic status as a member of *Termitomyces.*

Colour is very useful in delimiting the species of this genus, but it varies enormously probably due to different pigments they contain. Further studies are thus recommended to determine the pigment responsible for a certain suite of colour in order to make it a more stable distinguishing trait. Micro-morphological characters of *Termitomyces* species provide little information for species delineation as noted in this study which is in agreement with Härkönen *et al*.1995, Bother and Eicker (1991), except for a taxonomically doubted *T. citriophyllus* species whose dimension diverged from the rest members of the genus. Usually *Termitomyces* species are known for having basidia which are tetra sterigtimate bearing tetra basidiospore (Heim, 1942; 1977; Bels and Pataragetvit, 1982; Singer, 1986). However, the presence of untetra basidiospore in *T. umkowaan* was an exception noted for the first time in this study (Figs 5a, b), thus re defining the genus circumscription is essential.

CONCLUSION

Although macro-micromorphological characteristics provide enough information for most of *Termitomyces* species delimitation they fail in case of more closely similar species like *T. striatus* and *T. aurantiacus* which require more investigations using molecular characters. Some of the characters used have high taxonomic information in delimiting the species while others have none. Micromorphology provides the least information in delimiting the species while the tetra basidiospore circumscription of the genus is ambiguous. While the authenticity of the characters used in distinguishing *Termitomyces* species revealed some unuseful character, the study also showed that there still more useful characters yet to be revealed, while ascertaining the taxonomic status of some taxa and delimiting more similar species without molecular characters is impossible.

ACKNOWLEDGEMENTS

The authors are grateful to the Germany embassy that sponsored field work for sample collection, the Sida-SAREC project that sponsored data analysis at the National herbarium Cryptogamie (PC), France, and the curators of the PC for their collections.

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