Ecotypes or Genotypes? The Status of the Currently Recognized Infraspecific Taxa of *Acacia tortilis* (Forssk.) Hayne Growing in the Red Sea Hills, Sudan and Egypt

Howaida Faisal AbdElRahman

Thesis for PhD degree at the University of Bergen
2007

"We seek the truth, and will endure the consequences"

Charles Seymour

To my parents and to the memory of Prof. Nils Skaug

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Contents

Abstract

This study focuses on the *Acacia tortilis* species complex and the taxonomic relationship among its currently recognized infraspecific (*A. tortilis* ssp. *tortilis*, ssp *raddian*a and ssp. *spirocarpa*) in the Red Sea Hills of North-East Sudan and South-East Egypt. The morphological features used in floras to differentiate among these taxa were examined on a total of 520 specimens, collected from 33 localities. It was found that there is an association between the morphological characters and their immediate environment. Of 158 specimens subjected to molecular analysis 106 showed no agreement between classes formed using morphological and / or molecular data. In spite of the great genetic polymorphism among all the 158 specimens, there was no significant difference among the three putative *A. tortilis* sub-taxa. Genetic diversity among the samples studied seems linked to restricted gene flow among populations, but a positive correlation was found between the genetic variation and the physical distance between *A. tortilis* localities studied. Discriminating morphological characters of *A. tortilis* such as crown shape and growth form seem to be a result of traditional human management activities such as animal browsing and pollarding at different life stages. The currently recognized infraspecific taxa of *Acacia tortilis* in the Red Sea Hills, Sudan and Egypt appear to be ecotypes rather than genotypes, and accordingly the *A. tortilis* complex in the Red Sea Hills of Sudan and Egypt comprises only one species i.e. *A. tortilis*.

Preface and Acknowledgments

This thesis, entitled

"Ecotypes or Genotypes? The Status of the Currently Recognized Infraspecific Taxa of *Acacia tortilis* (Forssk.) Hayne Growing in the Red Sea Hills, Sudan and Egypt",

is submitted in partial fulfilment of the requirements for the degree of PhD at the Faculty of Mathematics and Natural Sciences, University of Bergen, Norway. The Quota program, as a part of the Norwegian State Lånakassen fund, International Office, the Biology Department and the Faculty of Mathematics and Natural Sciences, Nile Basin Research Program, University of Bergen are thanked for their financial support. Field work and laboratory expenses were supported by Olav Grolle Olsens legat, Felt og seminarstøtte (Biology Department, UiB), the Norwegian State Lånekasse and the Ecological and Environmental Change Research group (Biology Department, UiB).

The thesis comprises two parts. **Part A**: (Synthesis) gives a general introduction and background for the work, brief reviews of the material and methods and the results, followed by the overall conclusion and proposals for future work. **Part B**: (original papers) consists of four original papers through which the aims of this thesis project have been achieved.

I would like to express my gratitude and thanks to my supervisor, Assoc. Prof. Knut Krzywinski, for introducing me to this project and to his research group and for his supervision and for introducing me to Prof. Richard Holton Pierce from whom I learned a lot. I would like to extend my thanks to my co-supervisor Dr. Stefan Ekman for introducing me to the laboratory techniques essential for my work and for advice about running the analysis. My thanks also go to members of Environmental Change Research group (Biology Department, UiB) for their help and advice with my research.

My sincere appreciation goes to all those helped me during my fieldwork. In Egypt, the field work was part of a project in the Eastern Desert during which I benefited from collaborating with Gidske Andersen and Jonatan Krzywinski. I express my gratitude to Mr. Mohamed Fouda, head of the Egyptian Environmental Affairs Agency, for facilitating the permission to work in restricted areas, to the staff members of the St. Katherine Protectorate for their informative discussion about the technique they are using to conserve the *Acacia tortilis*, and to people in Marsa Allem for their hospitality and especially to Mahmoud Bashir Eisa and Salim AbdElGadir Sadallah for driving us and guiding us to targeted localities. In Sudan, I offer sincere thanks for the logistic help I received from the UNDP, Sinkat, with special thanks to Taj Elsir Kalai and O'Nour for arranging transportation and logistic support needed during the field work. I appreciate the guiding and driving of Ibrahim Tahir Bakash, Mohamed Hamid and Tahir. My sincere thanks go to the family of Mr. Ali Shash and Mr. Babiker Mohamed Ali for their hospitality during my stay in the Eastern Sudan.

My thanks too to people at the Botany Department and the Faculty of Forestry, University of Khartoum, for providing literature about the Acacia in Sudan and for discussing my research and its importance.

My sincere thanks go to all those who made my stay less lonely in Bergen. There is no space to mention all, but my special thanks go to Prof. Nils Skaug and Dr. Anne-Maria Skaug. In this connection I thank Mohamed Babiker for always being around, listening to all my sufferings, for his friendship, encouragement and technical support during the writing of my thesis. I appreciate the encouragement and support I am getting from my uncle Salah H ElHag, so thanks a lot.

But those really behind this research seeing the light are my parents, my brother and my sisters. I am so grateful for your love, encouragement, and prayers and for patiently waiting years for this thesis to be finalized. I wish them health and happiness and dedicate this thesis to them.

Howaida F. AbdElRahman

List of papers

I. Howaida F. AbdElRahman, Knut Krzywinski. **Environmental effects on morphology of *Acacia tortilis* group in the Red Sea Hills, North-Eastern Sudan and South-Eastern Egypt**.(Accepted: Forest Ecology and Management)

II. Howaida F AbdElRahman, Knut Krzywinski, Richard H Pierce. **Heterogeneity in group clustering of *Acacia tortilis* of the Red Sea Hills in Sudan and Egypt using morphological and molecular data**. (Revision with reviewers BMC Ecology).

III. Howaida Faisal AbdElRahman, Knut Krzywinski and Stefan Ekman. **Genetic Diversity and Population Differentiation in the *Acacia tortilis* Group of the Red Sea Hills, Southeast Egypt and Northeast Sudan**. (Manuscript to be submitted).

IV. Knut Krzywinski, Gidske L. Andersen, Howaida AbdElRahman. **Morphological diversity in Acacia tortilis – the effect of environmental factors, human use and management.** (Manuscript to be submitted).

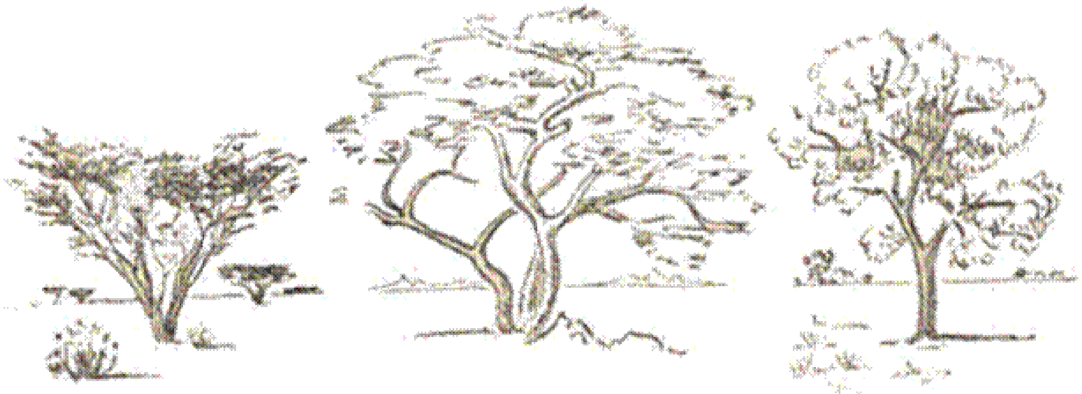
Introduction

Background

Leguminous plants are among the most important plant groups in the subtropical deserts. They, generally, provide protein and oil to humans and animals and fix atmospheric nitrogen, and are thus of considerable economic, ecological and biological interest [1-4]. In tropical Africa and Western Asia, the genus *Acacia* is characterized by its sharp thorns that protect the plants against the heavy browsing and differentiate them from the Australian acacias [5-9]. The vegetation of the arid land in North Africa and the Near East (for the worldwide distribution of arid land see Appendix A) is characterized by being poor in species diversity. Among the species that are found there is the *Acacia tortilis* (Forssk.) Hayne, which represents a common perennial species. It is widely distributed in the arid and semiarid areas of Africa, namely Senegal, Somalia, South Africa, in countries along the Sahara, in Israel, in southern Arabia and in Iran. *A. tortilis* is a drought-enduring species [10] with the ability to grow in habitats with an annual rainfall below 40 mm, with temperatures reaching up to 50° C and at altitudes ranging from 1 to 1000 meters above sea level. It is, however, frost tender [11]. It is tolerant to nearly all soil types but favours alkaline soils and cannot withstand waterlogging. Pollination is mainly by bees, ants and flies [12]. It is capable of fixing nitrogen and can stabilize sand dunes [13-17]. Powered by hydraulic lift, controlled by its deep-rooting system, it can extract water from deep subsurface resources and thrives where other co-existing plants failed to perform [18]. It is capable of creating a favourable micro-environment under its canopy and increases understorey plant production. In the arid regions of North Africa, it is the main source of animal fodder and of shade for nomads and desert-dwellers.

In Africa there are four infraspecific taxa of *A. tortilis* reported, *A. tortilis* ssp *raddiana*, ssp *tortilis*, ssp *spirocarpa* and ssp *heteracantha* [19, 20]. It remains unclear whether to classify them as subspecies of *A. tortilis* or as species in their own right [9, 17, 21]. In the following these four taxa are collectively referred to as the *A. tortilis* complex. While the distributions of the first three subspecies overlap in the area where they are growing, the ssp *heteracantha* is geographically separate and found growing in the Southern part of Africa. These areas of distribution are: subsp. *heteracantha* (South Africa, Mozambique, Zimbabwe, Botswana, S. Angola, Namibia and Swaziland); subsp. *spirocarpa* (East Africa and the horn of Africa: Sudan and Ethiopia southwards to Zimbabwe, Botswana and Namibia); subsp. *raddiana* (Senegal, Sudan, Somalia and Kenya and extending through Egypt to Israel, Jordan and Saudi Arabia); and subsp. *tortilis* (Egypt south to Sudan and Somalia extending east to Arabia). The variations in the architecture of these subspecies, expressed in their various growth-forms, are the main characters that differentiate them [19, 20, 22-25]. Ecological factors, including human utilization and animal browsing, play, however, a great role in forming the architecture of plants growing on arid land [4-7, 26-29]. In spite of the fact that the *A. tortilis* complex has a wide geographic distribution, very little is known about how the varying environmental factors along spatial gradients affect its morphology, *i.e.* their implications for taxonomy at the subspecies level. As a result, different floras give overlapping general morphological descriptions of *A. tortilis* [23, 30, 31](for detailed descriptions see Appendix B) as well as overlapping morphological descriptions of the subspecies [19, 22-25, 30, 32-34] (for detailed descriptions see Appendix C). It is, therefore, very difficult to identify the subspecies of *A. tortilis* in the field or in herbarium specimens. Already in 1930, Crowfoot raised the question of how *A. tortilis* should be classified [24]. He based his description of the three Acacias (*A. spirocarpa*, *A. spirocarpa* var *major* and *A. tortilis*) (Fig. 1), known as Samr and Seyal, on a translation of Schweinfurth's *Acacien Arten* "In situ: **Aufzahlung und Beschreibung der Acacien Arten des Nilgebiets. Dr. G. Schweinfurth**". He drew attention to the fact that the specimens of *A. spirocarpa* var *major* collected in the Red Hills of Sudan, were identified at Kew as *A. spirocarpa*, and that *A. tortilis* specimens collected at the Blue Nile in Sudan and elsewhere were also identified at Kew as *A. spirocarpa*, and that in fact no specimens of *A. tortilis* from the Sudan were found or reported either at Kew or at the British Museum. Crowfoot's problem has remained unclarified [9, 19, 21], and it is from this confusion and the inconsistency of identifying *A. tortilis* that the idea for the current research emerged.

This study focuses on the *A. tortilis* that is found growing in the Red Sea Hills regions of Sudan and Egypt (cf. study area below) in order to understand factors underlying the diversity in the morphological characters exhibited in the *A. tortilis* complex. To keep the process of identifying samples in the field consistent, only floras from Egypt and Sudan were consulted.



A. spirocarpa Hochst *A. spirocarpa* var *major* *A. tortilis* Hayne

Fig. 1. Three Acacias know as Samr and Seyal. Source Crowfoot 1930.

Objectives

The study was initiated to determine to what degree the *A. tortilis* subspecies are taxonomically related and whether what have been classified as subspecies are ecotypes rather than genotypes. Initially, this relationship was to be examined by incorporating morphological, environmental, molecular and chemical data, but chemical analysis was excluded from this study after the molecular analysis yielded sufficient interpretative results to permit an understanding of this taxonomic entity. Therefore, the **broad objective** of this study is to promote our understanding of the genetic variability, the morphological variation in the morphology, and the taxonomical relationship among the *A. tortilis* infraspecific taxa reported in the Red Sea Hills of North Eastern Sudan and South Eastern Egypt and thereby to explain the nature of the diversity of these subspecies. This broad objective is realized through a series of four interconnected studies that address specific issues arising from this objective which were presented in individual publications. The **specific issues** are:

- To examine the influence of environmental variables and spatial variation, on the expressed morphology of the *A. tortilis* samples studied.
- To see if the classes formed using the morphological and molecular data from the same individuals separately converged.

- To infer levels of gene flow, population structure, and the correlation between structure and current taxonomy in *A. tortilis* along a distinct environmental gradient in the Red Sea Hills of Sudan and Egypt.
- To examine how human use of Acacia may affect growth forms in different life stages.

Study Area

Broadly defined, the study area lies in the South-Eastern part of the Sahara [35]. The Sahara as we see it today is the result of a process of desiccation that began abruptly at about 5500 BP and changed an open savannah forest into desert vegetation [36-39]. Specifically, the study area lies in the Red Sea Hills regions and comprises two different regions: North-Eastern Sudan (between 18º 40 and 19° 11N latitude and 35º 54 and 37° 5E longitude) and South-Eastern Egypt (between 24° 9N and 25° 5N latitude and 33° 58E and 35° 3E longitude) (Fig.2). On the aridity index, the study area is classified as arid land in Africa [40], but local climates still persist due to variation in temperature and precipitation [41, 42]. The Egyptian localities fall within the range of hyperarid deserts, while the Sudanese localities are in this context less arid.

Localities studied

The current study, as part of a multidisciplinary Expedition to the Eastern Desert (February – April 2003), included visiting a total of 33 localities both in Sudan and Egypt. But for the purpose of the above mentioned aims only 25 localities were selected (Egypt = 13 localities <February – March 2003> and Sudan = 12 localities <April 2003>) (Table 1). The Sudanese localities were revisited in January – February 2005 to collect samples for chemical analysis. According to UNESCO [35], the Egyptian localities lie in sites characterized by uniform climatic conditions, i.e. they are hyperarid and characterized by having hot summers (mean of hottest month is 20° -30° C), mild winters (10° - 20° C) with winter rainfall (annual rainfall ≤ 75 mm). By contrast, the Sudanese localities lie in sites with two different climatic conditions, both of which are characterized as arid. Both these climatic conditions are characterized by having hot summers (mean hottest month is more than 30° C) and warm winters; but some localities have both summer and winter rain while the others have summer rain only.

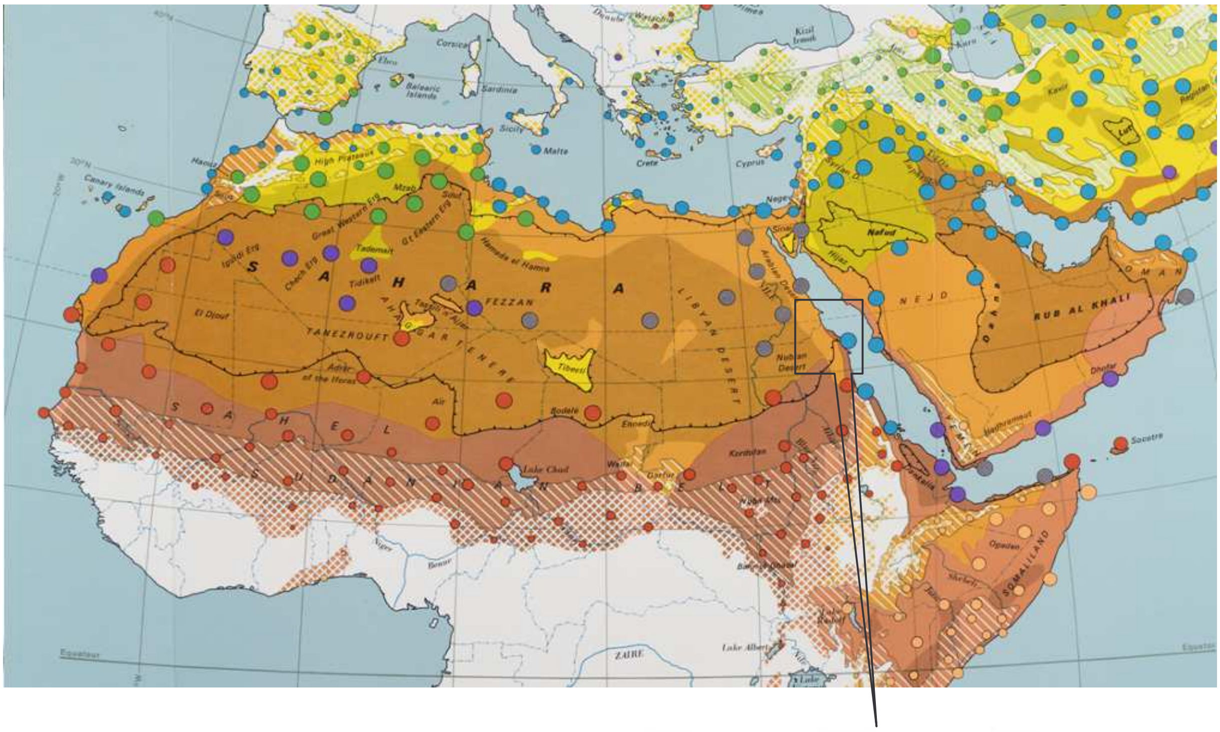
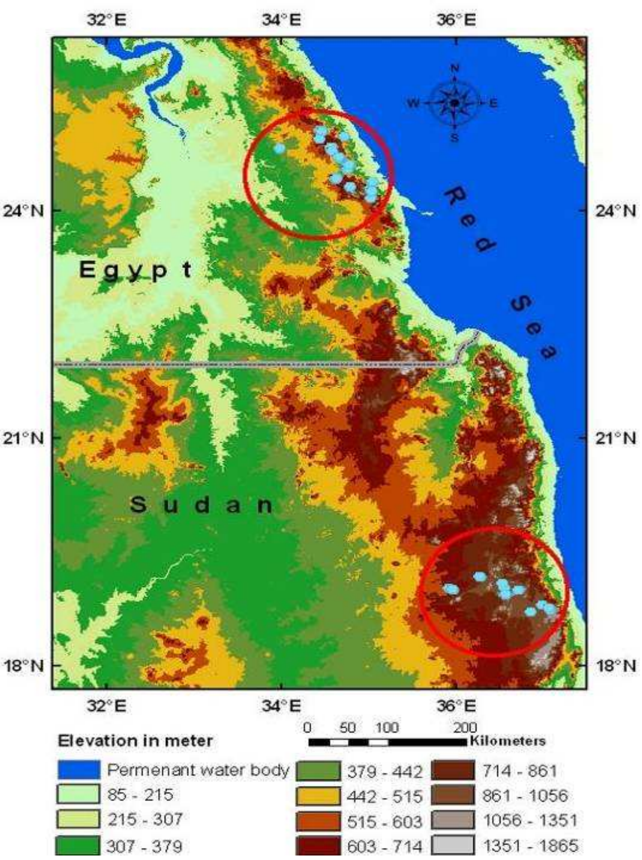


Fig. 2. Study area. Maps showing the location of the study area which lies in South East Egypt and North East Sudan. The topmost part of the figure is taken from a map showing the world distribution of arid land, source UNESCO. The lower part is a map based on a digital elevation model showing the localities studied (indicated by blue circles) in each region (red circles). For the whole world distribution of arid regions and the key to the colors of the upper map see appendix A



The localities studied experience a variety of local climates. These localities benefit from their adjacency to the Red Sea, the Red Sea Hills lying about 15 to 20 miles from the shore inland and running almost parallel to the coast (Fig.3) [43]; while they suffer from a lack of frequent rainfall and a great variation in their daily temperature. These climatic conditions in the Red Sea Hills are a product of (1) location within the tropical zone; (2) the presence of the Red Sea as an adjacent body of water, and (3) the Red Sea Hills as an effective physical barrier [44]. However, the landscape of the Red Sea Hills can be divided into units defined by climate, geology, and geomorphology [45]. Among these units are the seasonal water courses (locally are known as khors or wadis, in other places referred to as dry river beds), the drainage system of which governs the distribution of the available surface and subsurface water resources. It is mainly along drainage lines and on the flood plains that perennial vegetation occurs [10]. Such water courses are characteristic of hot deserts and carry water only during floods.

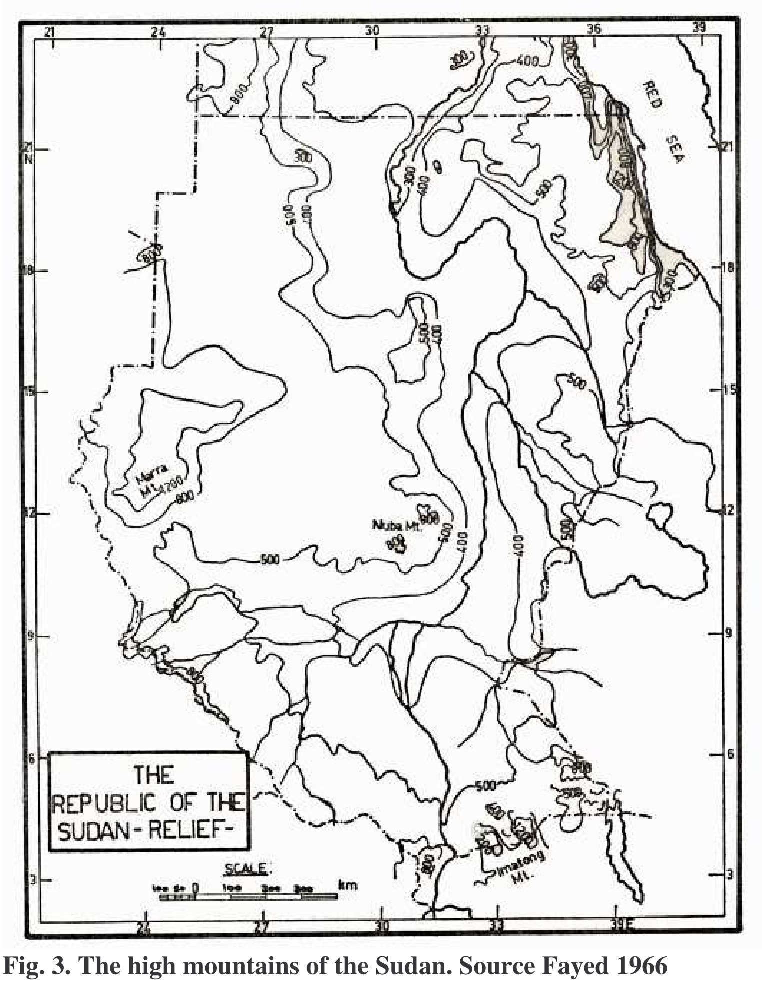


Fig. 3. The high mountains of the Sudan. Source Fayed 1966

The northern (Egyptian) localities experience less frequent summer rain and less influence of orographic rain from the sea than the southern (Sudanese) localities (Fig.4) [46]. The high mountains and plateaus in the south (Fig.3) act as an effective barrier to the climatic influence of air masses from either side of the watershed. This effect is, however, modified in places by lower altitude mountain passes that create corridors.

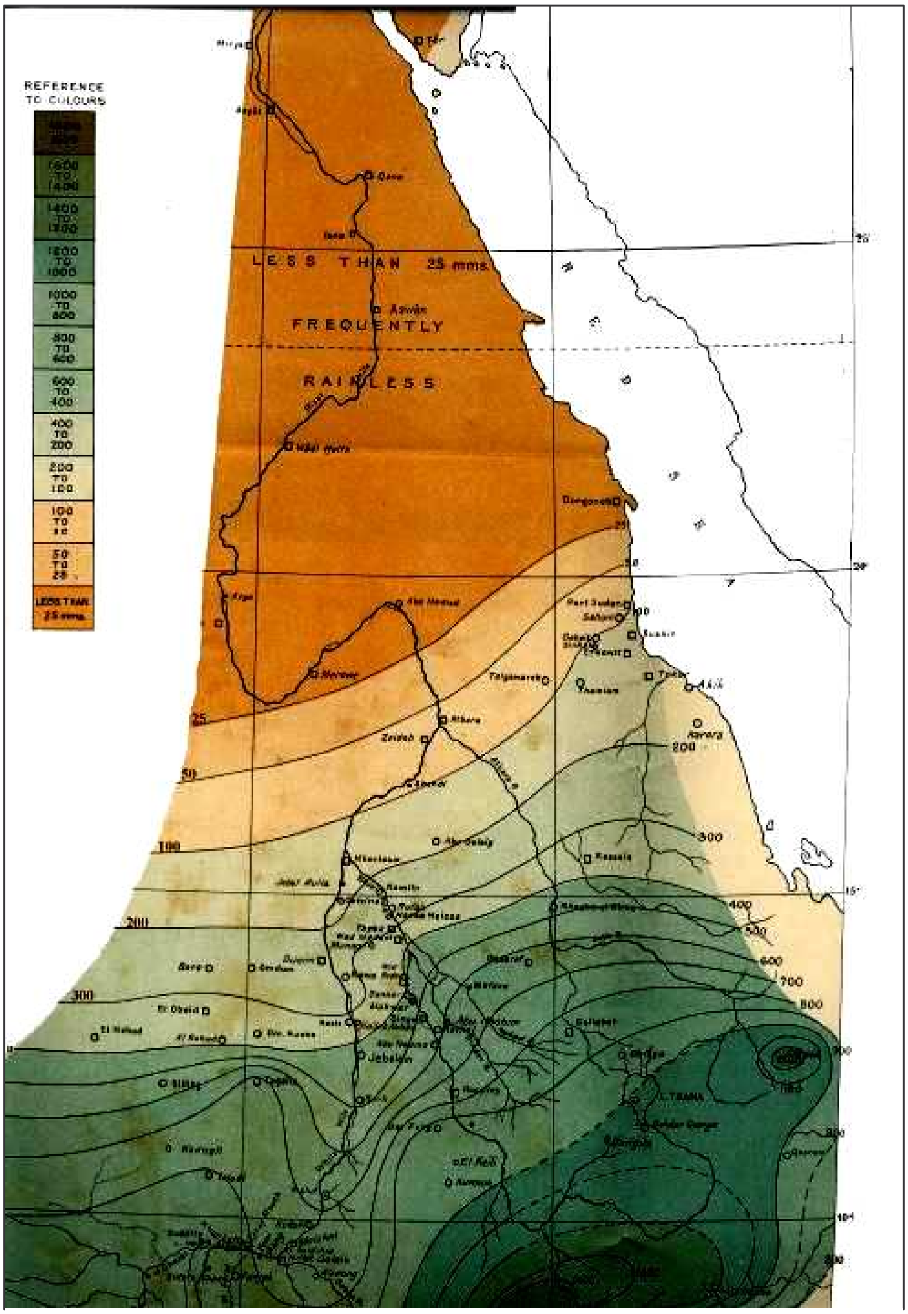


Fig. 4. Distribution of Annual rainfall in the study area. Source Ministry of public works, physical department, Egypt

Table 1.Names, altitude and geographical locations of the localities visited February – April 2003

Major region	*Locality name*	*Elevation average (m.a.s.l.)*	*Geographical locations*	
			E	**N**
Egypt	Lower Wadi Hulus	439	34° 38'	24 26'
	Wadi ElJimal 1	286	34° 45'	24° 33'
	Wadi ElJimal 2	285	34° 46'	24° 33'
	Wadi Nuqrus	304	34° 46'	24° 37'
	Wadi Sukkari	300	34° 43'	24° 59'
	Wadi Hanjaliyya	707	34 34'	24 50'
	Wadi Sartut 2	347	35° 01'	24° 17'
	Wadi Abu Ghusun	216	35° 02'	24° 23'
	Wadi Hulayfi	513	35° 02'	24° 15'
	Upper Wadi Nuqrus	600	34° 34'	24° 47'
	Mid Wadi Nuqrus	421	34° 40'	24° 42'
	West Wadi Hanjaliyya	565	34° 26'	24° 57'
	Wadi Muylhie	358	33° 59'	24° 49'
Sudan	Upper Beramio	1052	36° 58'	18° 47'
	Lower Beramio	920	36° 51'	18° 42'
	Upper Amat	1079	37° 05'	18° 44'
	Lower Amat	1018	37° 03'	18°46'
	Upper Hilayet	973	36° 43'	18° 59'
	Nubahweeb	883	36° 33'	19° 01'
	Upper Biramfi	926	36° 33'	18° 56'
	Dybadwat	852	36° 31'	19° 05'
	Lakatyeb	816	36° 17'	19° 10'
	Upper Sarara Agwampt	642	35° 58'	18° 59'
	Lower Sarara Agwampt	611	35° 55'	19° 01'
	Mid Sarara Agwampt	631	35° 57'	19° 00'

Materials and Methods

Each plant sample studied was recorded in the field. All samples, a total of 520 individuals, have been:

- morphologically described and recorded in the field; these morphological observations (see next section) were from the vegetative part of the plants since it was difficult to find floristic parts in all the samples. These records were similar to those used in the floras of the study area to differentiate between the *A. tortilis* sub-species [19, 22, 23, 25, 30, 32, 33].
- digitally photographed (Sony Cybershot 3.2 megapixles).
- geographically positioned (GARMIN GPS 12). Geographic coordinates were used to map the locations of the localities studied upon a digital elevation model to create the map of the study area (Fig. 2).

Of these 520 samples, 158 were subjected to the molecular testing.

Morphological data

The following characters were recorded in field.

- **Height of the plant**: measured in meters from the base of the stem at the ground level to the top of the crown;
- **Growth form:** either tree or shrub (Fig. 5);
- **Number of stems from ground level:** stem = 1, stem range from 2 to 5 or stem > 5 (Fig. 5);
- **Distribution of hairs when seen by the naked eye**: either no hair, only in the petiole or young branchlets densely pubescent.
- **The shape of the crown**: either round, flat, spread or undefined;
- **Spine shape seen in an individual plant**: spines are either straight or spines are = mixed, i.e., both straight and curved (Fig 6).

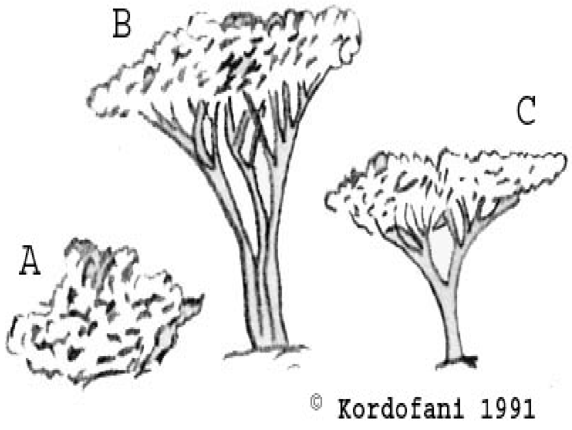


Fig 5. Illustration of the morphological variables. Growth form (A) Shrub; (B&C) Tree. Number of stems (B) three stems; (C) one stem

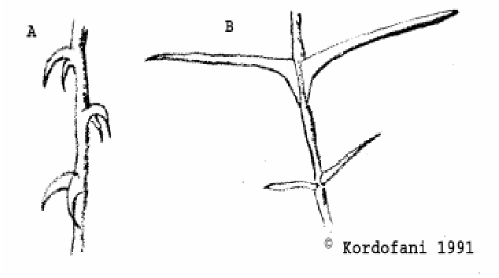


Fig. 6. Spine shape. (A) curve; (B) straight.

Environmental data

Three major environmental variables were recorded; two of them were further subdivided into three sub-variables. These were:

- **Position of the khor/wadi within the catchment**: either upper run, mid run, or lower run (Fig. 7 A). These reflect the size of catchments and represent the longitudinal distribution of the plants with respect to the water distribution in the khor / wadi.
- **Location of the plant within its locality**: either in mid khor, at the khor bank, or on the hillside (Fig. 7B). These reflect distance from main drainage line and represent the cross-sectional structure of the catchment;
- **Elevation of the locality:** measured in meters above sea level (m.a.s.l.). The stated elevations (Table 1) are averages of the elevations of the plant samples in each locality extracted from a freely downloaded Digital Elevation Model, source http://seamless.usgs.gov/, using ArcGis version 9.0.1 software.

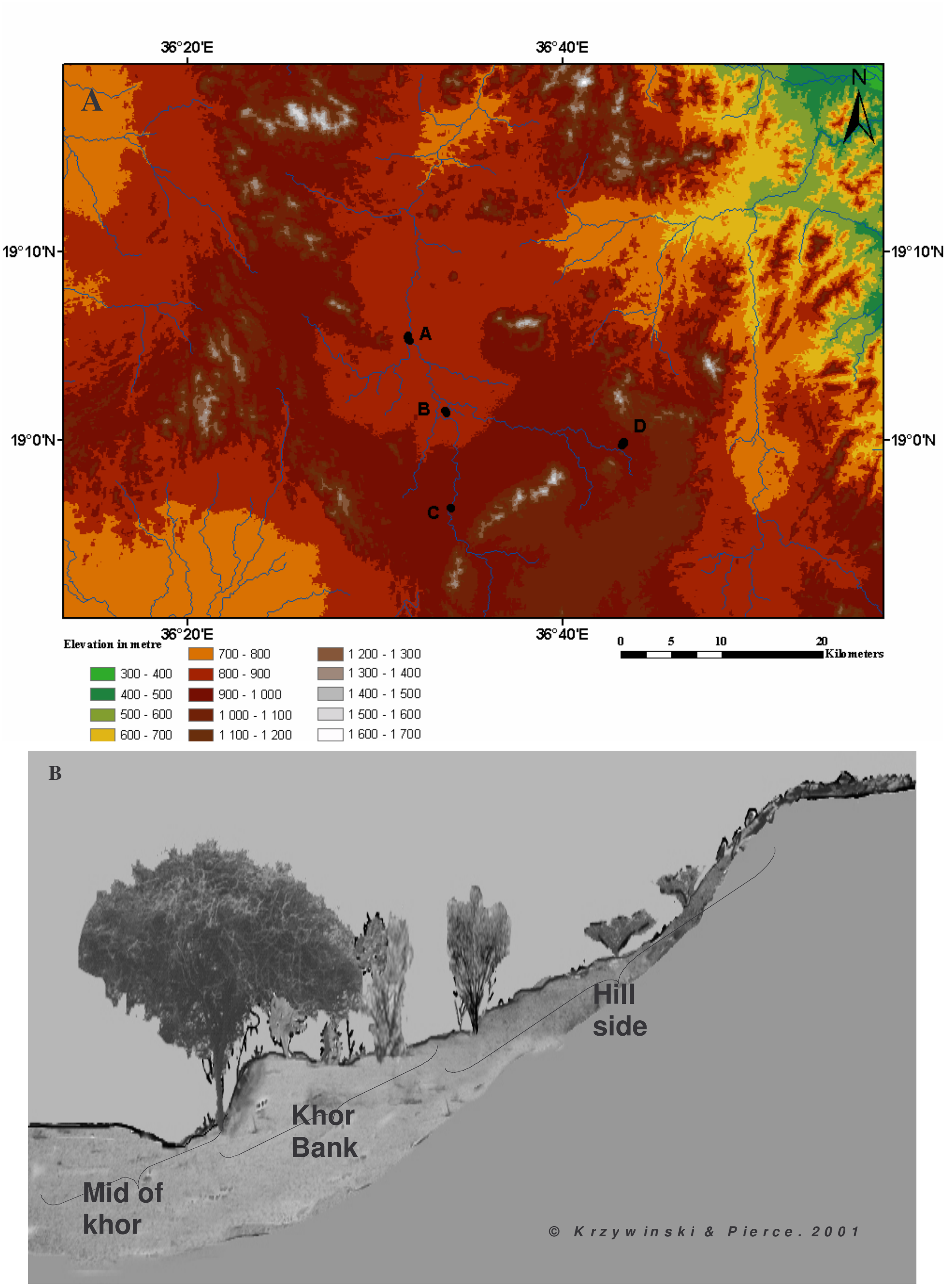


Fig 7. Longitudinal and cross-section catchment (khor/wadi): A) Map demonstrating the longitudinal structure of the catchment. In this illustration some of the Sudan's localities are shown to represent what is meant by the upper (C&D), mid (B) or lower

Sample collection

Acacia tortilis leaves were collected in the field, wrapped in gauze, labelled and placed in plastic bags filled with silica gel [47]. The silica gel was changed as often as the colour changed indicating the hydro-saturation of the silica gel. Then they were shipped to The University of Bergen, Biology Department where the laboratory analysis for this study was performed.

Sub-taxa identification

As stated above (cf. Appendix C), the characters that have been used in the various floras to identify the sub-taxa of *A. tortilis* overlap and are inconsistent. However, for the current study the three reported sub-taxa in the study area needed to be identified so as to verify their eco- and / or genetic relations. Therefore criteria based on what is presented in Appendix C, using floras of Egypt and Sudan, is applied to differentiate between the *A. tortilis* sub-taxa as needed in this study. These were as follows: plant characterized with being a (Fig.8):

- **tree, having one stem, a round crown and being glabrous** was identified as spp. *raddiana*.
- **small tree or shrub, having more than 5 stems, a flat crown and hair distributed over the young branchlet** was identified as spp. *tortilis*.
- **tree, having 2-5 stems, a flat-spreading crown, and with hair either at the petiole or over the young branchlet** was identified as spp. *spirocarpa*.
- When these characters overlapped the plant was identified as unclassified.

Fig 8. ***A. tortilis*** **subtaxa as identified on field based on descriptions in Appendix C. The subtaxa are ssp.** ***raddiana*****, ssp.** ***spirocarpa*****, and ssp.** ***tortilis*****, respectively.**

Molecular data

DNA extraction and RAPD-PCR amplification

Rapid amplified polymorphic DNA (RAPD) was the genetic marker used to study DNA patterns of *A. tortilis* [48]. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN) following the manufacturer's protocol. Details of the procedures are described in full detail in paper III.

Statistical Analysis

Various statistical approaches were applied according to the sub-objectives of the current research.

- To study the relation between the different morphological patterns that the *A. tortilis* samples expressed and their intermediate environment: CANOCO for Windows version 4.5 was the software applied and Redundancy Analysis (RDA, Monte Carlo permutation test was set at 499 and significance level p-value was ≤ 0.05) was performed. The number of samples studied was 520. The details of the analysis were stated in Paper I. Data analyzed:
    - ➢ Morphological variables were all those stated above.
    - ➢ Environmental variables were all those stated above.
    - ➢ Supplementary variables were the sub-taxa of *A. tortilis* (Fig. 8), but when the characters overlapped then the plant was recorded as unclassified.
- To assess taxonomic relationships in the *A. tortilis* complex in the Red Sea Hills in Sudan and Egypt, using both morphological and molecular data. XLSTAT software was employed for cluster analysis using various indices suitable for the data. The number of samples studied was 106. Details of the analysis are described in Paper II. To fulfil the aim of this assessment, binary data (presences / absence data) was analysed. Data analyzed:
    - ➢ All morphological variables were used with the exception of the plant height. Height was excluded because it was recorded as a continuous variable in meters. Data need to be kept consistent with the molecular variables (presence / absence of bands).
    - ➢ DNA data.

- To infer levels of gene flow, population structure and the correlation between structure and current taxonomy in *A. tortilis* along a distinct environmental gradient along the Red Sea Hills of Sudan and Egypt, analysis of molecular variance (AMOVA) and Mantel test were applied using Arlequin version 3.1. The numbers of samples analyzed was 158. Details of the analysis are described in paper III. Data analyzed:
    - ➢ DNA data.
    - ➢ Sub-taxa identification.
- To examine how human use of *A. tortilis* may have affected growth forms in different life stages. The effects of the environmental conditions, human utilization and animal browsing were studied separately and combined with the various growth-forms in different life stages. Details of these analyses are described in paper IV.

Results

The main results that composed this study were:

➢ **Paper I: Howaida F. AbdElRahman, Knut Krzywinski. Environmental effects on morphology of *Acacia tortilis* group in the Red Sea Hills, North-Eastern Sudan and South-Eastern Egypt**.

In this paper we studied the correlation between the immediate plant environment and the expressed morphological characters of *A. tortilis*. First we analysed the data from the two regions, i.e. Sudan and Egypt, as one data set. Thereafter we ran a similar analysis but with data from each region separately. A correlation between the immediate environment and the expressed morphological patterns of *A. tortilis* was found. Elevation, above sea level, was the only significant response variable when the 520 samples were studied as one set of data. Higher hair density was associated with plants growing in Sudan. However, when only data from Sudan was studied separately the statistically significant response variables were elevation, hillside and lower part of the catchment. In the Egyptian data set the statistically significant response variables were elevation, khor bank and the upper, mid, and lower part of the catchment.

- **Paper II: Howaida F. AbdElRahman, Knut Krzywinski, Richard H. Pierce. Heterogeneity in group clustering of *Acacia tortilis* of the Red Sea Hills in Sudan and Egypt using morphological and molecular data.**

In this paper we were trying to find out if there is any agreement between classes formed using morphological and / or molecular data from the same plants. Using the same clustering indices, no concordance was found between the classification of *A. tortilis* using morphological and / or molecular data and the current taxonomy of *A. tortilis* used in the flora. Morphologically different individuals showed similar DNA patterns (Fig. 9).

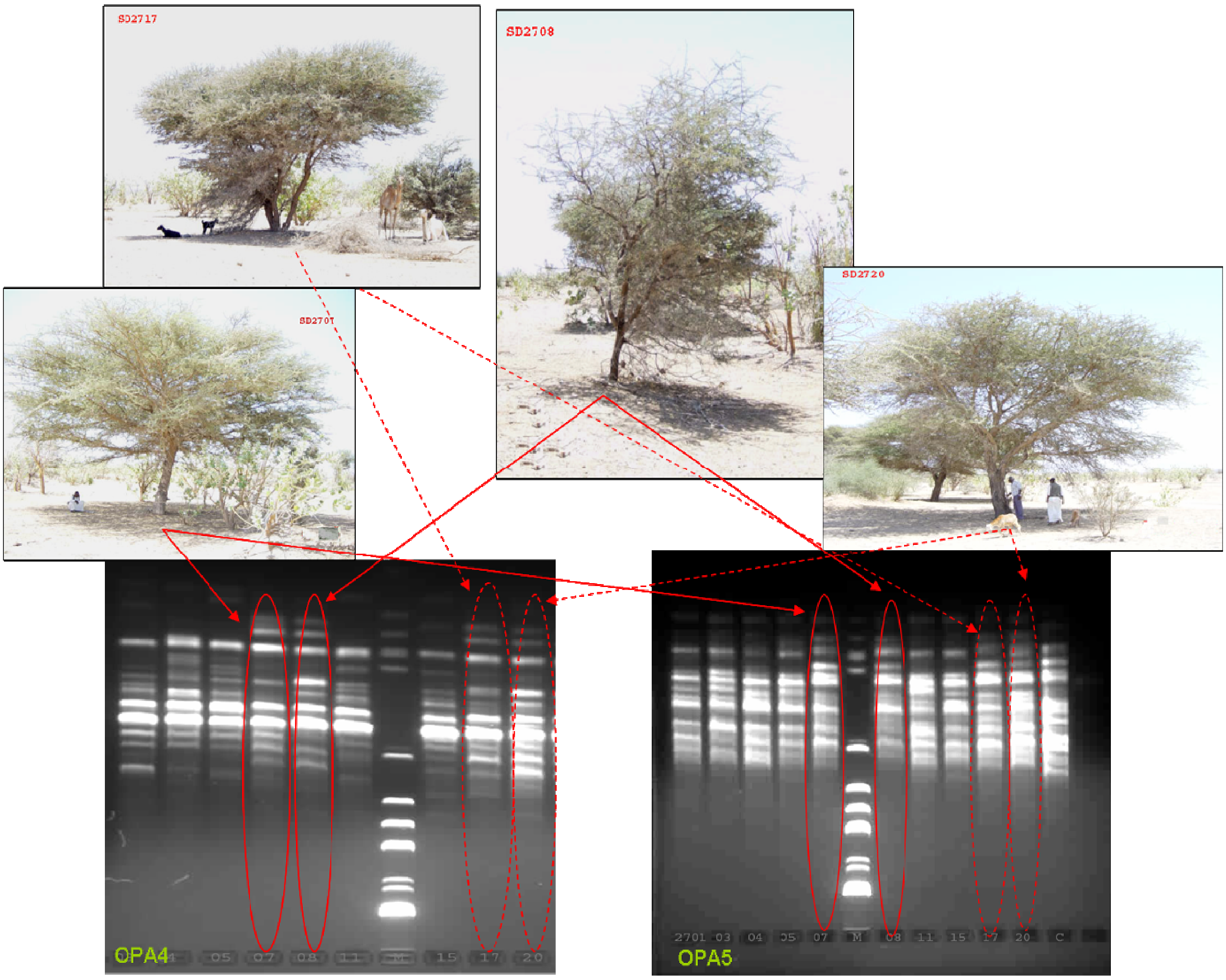


Fig. 9. Morphologically different but genetically similar individuals of *A. tortilis* samples collected in Sudan. Arrows (dotted or solid) indicate similarity in morphological classes.

- **Paper III. Howaida Faisal AbdElRahman, Knut Krzywinski and Stefan Ekman. Genetic Diversity and Population Differentiation in the *Acacia tortilis* Group of the Red Sea Hills, Southeast Egypt and Northeast Sudan**.

From paper II above, the high level of genetic polymorphism in *A. tortilis* from the Red Sea Hills regions of Sudan and Egypt couldn't explain the diversification of their morphology. In this paper we studied factors underlying the genetic diversity revealed by these *A. tortilis* specimens. The exact test of population differentiation showed that there was no infraspecific differentiation among the *A. tortilis* individuals studied. A high level of genetic polymorphism was distributed among *A. tortilis* individuals growing in one locality. A positive correlation was found between the genetic variation and the physical distance of all the *A. tortilis* populations studied in Egypt and Sudan. These results indicate that the gene flow between populations is restricted and that there is a significant isolation-by-distance between populations.

- **Paper IV. Knut Krzywinski, Gidske L. Andersen, Howaida F. AbdElRahman. Morphological diversity in *Acacia tortilis* – the effect of environmental factors, human use and management.**

In this paper we present the effect of utilisation on *A. tortilis* growth forms which has been poorly acknowledged in existing literature. We show that there is no clear distinction between the growth forms of subspecies and explain how the main growth forms and intermediate forms can have resulted from a combination of local climate, human use, browsing and pollarding. Crown shapes are among the main characters that have been used to differentiate between the *A. tortilis* subspecies, but pollarding and browsing play an important role in the final form of a crown shape. Another type of growth form is the number of stems from the ground level, but in early stages trees are bushes or multistemmed trees with flat crowns as a result of browsing. Hitherto these forms have been referred to as characteristic of the *Acacia tortilis* ssp. *tortilis*. Hairs on pods and vegetative parts have been cited as characteristic of the *Acacia tortilis* ssp. *spirocarpa*; but at certain locations, particularly in Sudan, moisture conditions favour the development of hairs on those parts.

Conclusion

This study was initiated to clarify our understanding of the taxonomical relationship(s) in the *Acacia tortilis* complex growing on the Red Sea Hills of South-East Egypt and North-East Sudan using molecular analysis. Prior to this study that complex has been referred to as a set of four subspecies of *A. tortilis* (ssp *heteracantha*, ssp *tortilis*, spp. *raddiana* and spp. *spirocarpa*), and the floras differentiated between them using their morphological diversification without acknowledging the effects of the *environment*, *plant management* and *anthropogenic activities* on the growth forms or using molecular tools to study their taxonomical relationship *genetically*.

Genetically, the main conclusions are: no significant difference between putative subspecies of *A. tortilis* in the Red Sea Hills of Sudan and Egypt; nor did either their genetic or morphological diversity explain their current taxonomy to the subspecies level as presented in the various floras. Their genetic diversity can be explained by gene flow being restricted between the *A. tortilis* populations in the study area and occurring mainly at relatively short distances, i.e. by isolation-by-distance.

As regards the effects of the *environment*, *plant management* and *anthropogenic activities* on the growth forms, the main conclusions are: ecological factors including human utilization and management and animal browsing are the major factors underlying the various growth forms of the *A. tortilis* complex in the Red Sea Hills of North-East Sudan and South-East Egypt. Combinations of these factors suffice to explain the morphological diversity within the *A. tortilis* complex.

The overall conclusion of this thesis is that the *A. tortilis* specimens studied are ecotypes.

The genetic divergence of *A. tortilis* populations of the Red Sea Hills in Egypt and Sudan has implications for the management and conservation of the species. Collecting *A. tortilis* seeds randomly at one locality for replantation in another should be avoided if local genetic uniqueness is to be preserved.

Suggestions for Future Research

- This thesis has established the taxonomic unity of the *A. tortilis* growing in the Red Sea Hills of Sudan and Egypt. Genetically, it is a single species, and the floras must be revised accordingly. But to provide a complete and concrete updating of the taxonomy of *A.*

tortilis further research applying an approach similar to that of the current studies is needed and should include the following measures:

1. Increase the number of Egyptian samples and include more northerly localities, e.g. Sinai Peninsula.
2. Extend research to include *A. tortilis* along the whole entire moisture gradient in Africa.
3. In connection to point 2 above, include the fourth "subspecies" *A. heteracantha* from South Africa.
4. To corroborate that the difference in genetic variation among *A. tortilis* in Sudan and Egypt is due to gene flow restriction extend research to include an intermediate area between the two regions already studied. On area important to study is Gebel Elba (South East corner of Egypt at the Sudan frontier).
5. Include other isolated populations in the Sahara to study the differences over a wide area, e.g. Gebel Uweinat on the Sudanese–Egyptian–Libyan political border.
6. Study the relation between *A. tortilis* and other acacias to discern its potential value in a sustainable economy.

- The work reported in this thesis has exhibited many of the challenges for the use of molecular data as a useful tool to provide information needed to review and update floras, but for practical reasons molecular data can never fully replace morphology as a tool for taxonomy. The overall conclusion of this study confirms the importance of integrating ecological studies to establish the appropriate taxonomy for a given object of study. Morphological data is needed to understand the ecology of a species and therefore indispensable in evolutionary studies, for only by integrating both morphological and molecular data can a sound taxonomy be established.

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Appendices

Appendix A

World Distribution of Arid Land, UNESCO.

Appendix B

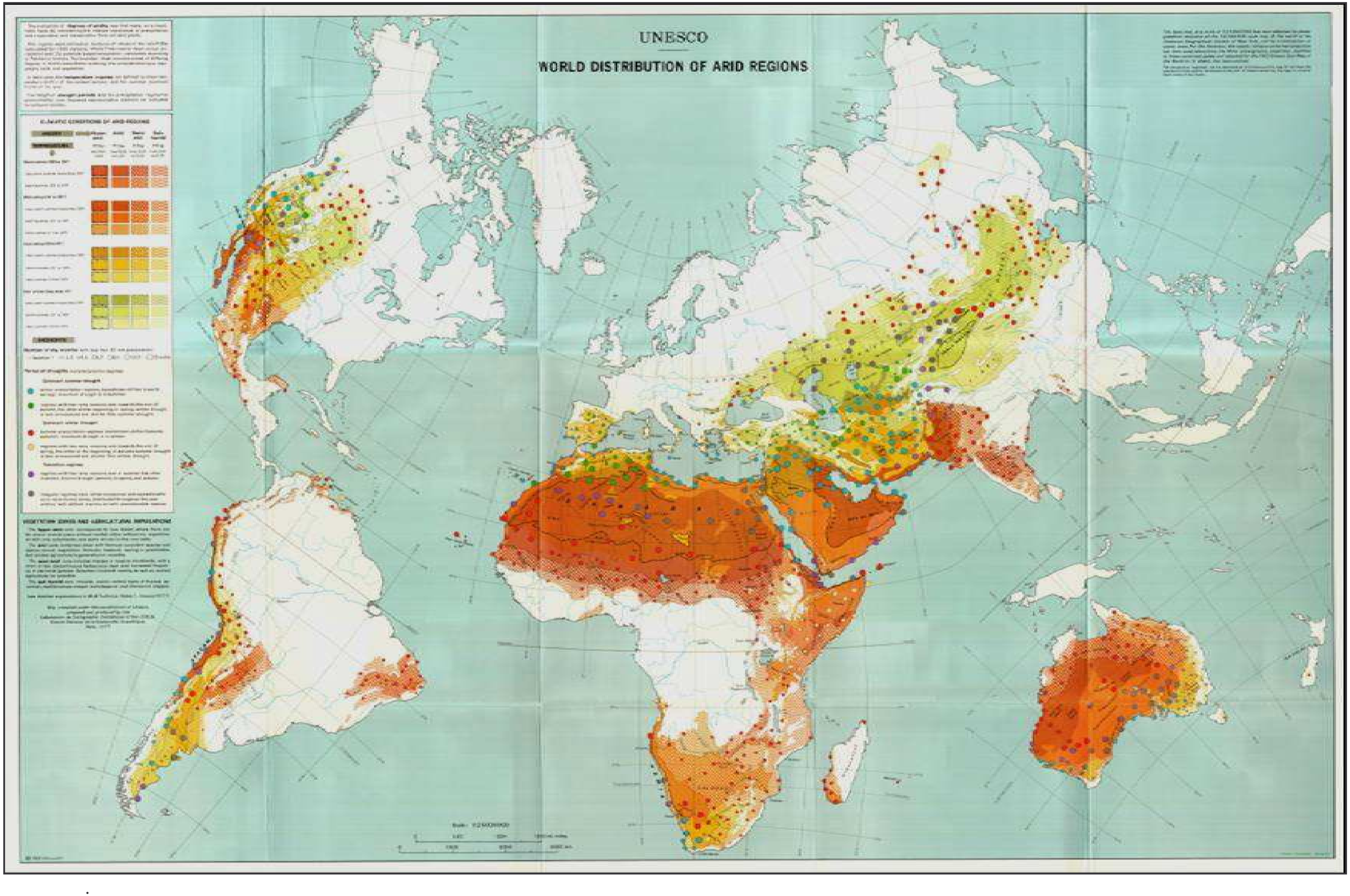
General description of *A. tortilis*. Empty cell indicate that the author(s) didn’t mention the concerned description.

Appendix C

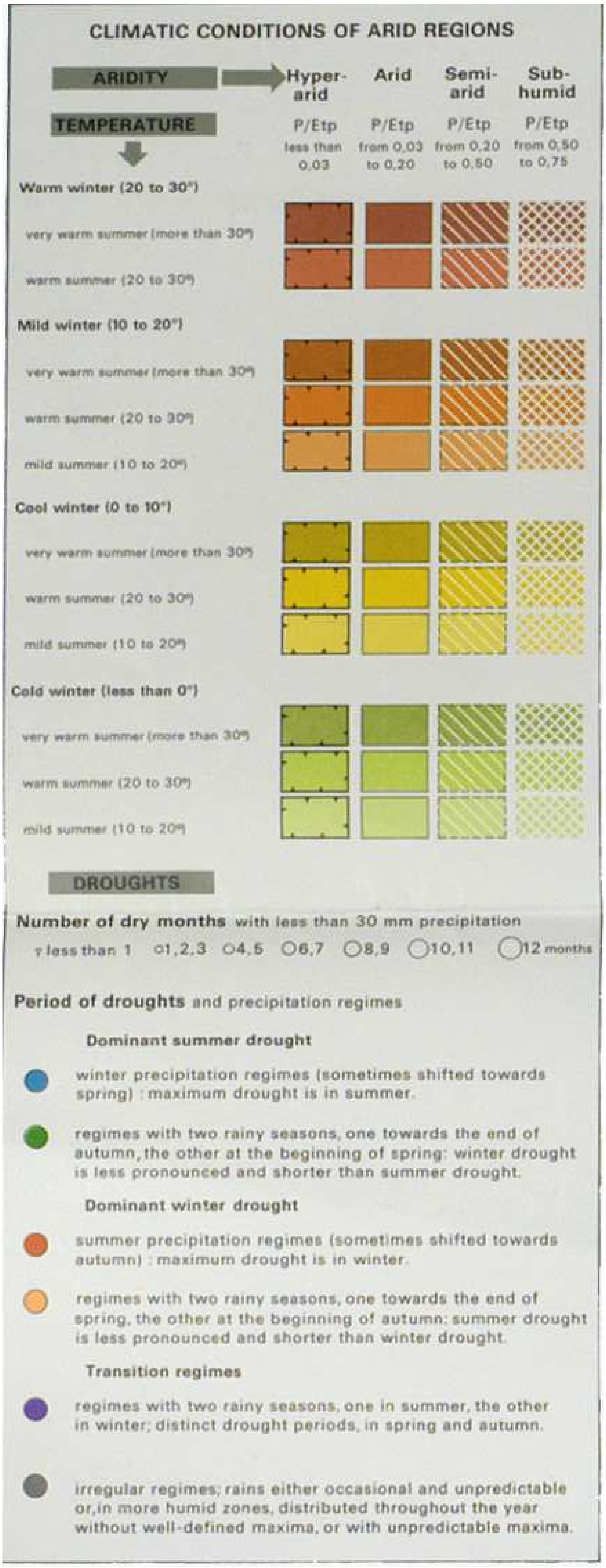
Characters used to differentiate between the various sub-taxa of *A. tortilis*. Empty cells indicate no description provided by the author(s).

Appendix A

World Distribution of Arid Land, UNESCO.


UNESCO
WORLD DISTRIBUTION OF ARID REGIONS

Key of the map



CLIMATIC CONDITIONS OF ARID REGIONS

ARIDITY →

TEMPERATURE ↓

	Hyper-arid P/Etp less than 0,03	Arid P/Etp from 0,03 to 0,20	Semi-arid P/Etp from 0,20 to 0,50	Sub-humid P/Etp from 0,50 to 0,75
Warm winter (20 to 30°)				
very warm summer (more than 30°)	■	■	▨	▦
warm summer (20 to 30°)	■	■	▨	▦
Mild winter (10 to 20°)				
very warm summer (more than 30°)	■	■	▨	▦
warm summer (20 to 30°)	■	■	▨	▦
mild summer (10 to 20°)	■	■	▨	▦
Cool winter (0 to 10°)				
very warm summer (more than 30°)	■	■	▨	▦
warm summer (20 to 30°)	■	■	▨	▦
mild summer (10 to 20°)	■	■	▨	▦
Cold winter (less than 0°)				
very warm summer (more than 30°)	■	■	▨	▦
warm summer (20 to 30°)	■	■	▨	▦
mild summer (10 to 20°)	■	■	▨	▦

DROUGHTS

Number of dry months with less than 30 mm precipitation

▿ less than 1 ○ 1,2,3 ○ 4,5 ○ 6,7 ○ 8,9 ○ 10,11 ○ 12 months

Period of droughts and precipitation regimes

Dominant summer drought

● winter precipitation regimes (sometimes shifted towards spring) : maximum drought is in summer.

● regimes with two rainy seasons, one towards the end of autumn, the other at the beginning of spring: winter drought is less pronounced and shorter than summer drought.

Dominant winter drought

● summer precipitation regimes (sometimes shifted towards autumn) : maximum drought is in winter.

● regimes with two rainy seasons, one towards the end of spring, the other at the beginning of autumn: summer drought is less pronounced and shorter than winter drought.

Transition regimes

● regimes with two rainy seasons, one in summer, the other in winter; distinct drought periods, in spring and autumn.

● irregular regimes; rains either occasional and unpredictable or, in more humid zones, distributed throughout the year without well-defined maxima, or with unpredictable maxima.

Appendix B

General description of *A. tortilis*. Empty cell indicate that the author(s) didn’t mention the concerned description.

General description of *A. tortilis*. Empty cell indicate that the author (s) didn't mention the concerned description

		Characters used in the description of A. tortilis						
		Growth form	**Number of stems**	**Branchlets**	**Leaves**	**Stipules/ Thorns / Spines**	**Inflorescence**	**Legume**
Authors	**Hassan 74**	Flat-topped tree		Pubescent	• Pinnae 7 -15 pairs. • Leaflets 3 - 10 pairs.	Spines mixed, some white and straight, others gray. Recurved small.	Flowers white	Pod yellowish brown, pubescent, spirally twisted, slightly constricted between the seeds
	ElAmin 90	• Small shrub or a tree, 12 m high. • Bark grey brown to yellow, smooth or fissured.	One to multi-stemmed plant		• Leaves 0.5 – 3 cm long • Pinnae 2-5 pairs, 0.2 -18 mm long • Leaflets 12-16 pairs, oblong linear	Stipules spinescent, hooked, 2-6 mm long, dark brown or straight or white or both 1 -10 cm long	• Inflorescence capitate, white on pubescent peduncles 1-2 cm long; involucel on lower half. • Flowers bisexual and male; sepals 1-3 x 0.5 mm, pubescent; petals 2 x 0.3 mm, dark-tipped; anthers free, 3.5 mm long, glandular.	• Fruit spirally twisted or coiled, constricted, flat to non compressed pods 5 - 15 x 0.2 – 1 cm, light green, yellow brown to pinkish; • seeds longitudinal in pod, elliptic, slightly compressed, 6 x 35 mm, olive green to red brown, smooth; areoles U-shaped, marginal; funicles 4-5 mm long, coiled
	Boulos 1999	Tree 5 -12 m		Glabrous or pubescent	• Pinnae 3 – 10 pairs • Leaflets 6-20 pairs, with a small gland under each pair, 0.5 -5 x 0.5 -1 mm, oblong, obtuse, glabrous or oppressed-hairy	Stipular spines in pairs, 2 -5 mm, mixed with straight spines to 10 cm	• Peduncles 0.5 – 3.5 cm, with involucel below the middle • Heads axillary • Calyx 1-2 mm • Corolla 1.5-2.5 mm • Whitish-yellow	Pod 3-8 x 0.6-1.2 cm, contorted or spirally coiled, constricted between seeds, longitudinally veined, glabrous or tomentose; seeds 4-7 x4-6 mm, compressed, the areole 3-5 x2-3 mm, brown, smooth.

Appendix C

Characters used to differentiate between the various sub-taxa of *A. tortilis*. Empty cells indicate no description provided by the author(s).

Characters used to differentiate between the various sub-taxa of *A. tortilis*. Empty cells indicate no description provided by the author (s)

			Characters used for identification of A. tortilis sub- taxa									
Author	**Sub-taxa**	**Synonymies**	**Growth form**	**Height in meter**	**number of stems**	**Young branchlets**	**Spines**	**Flowers**	**Leaves**	**Pods**	**Crown**	**Location**
Broun 1906	*tortilis*	*A. tortilis* Hayne	Obconical shrub or small tree				straight and curved					
	spirocarpa	*A. spirocarpa* Hochst.	large tree				straight					
Broun & Massey 1929	*tortilis*	*A. tortilis* Hayne	large shrub or small tree				straight and curved	flowers-heads globose, whitish	Pinnae 2-5 pairs; leaflet 8-12 pairs; linear-oblong	twisted		
	spirocarpa	*A. spirocarpa* Hochst.	large tree				straight	flowers-heads globose, greenish-white	Pinnae 4-10 pairs; leaflet 7-15 pair; very small	spirally twisted	flat	
Crowfoot 1930	*tortilis*	*A. tortilis* Hayne	tree	9 - 12	1	glabrous		pale yellow		glabrous	irregularly / roundish	
	spirocarpa	*A. spirocarpa* Hochst.	obconical shrub or small tree	0.3 - 6	many	pubescent	hooked & straight	small pale yellow		pubescent, twisted		

			Characters used for identification of A. tortilis sub- taxa									
Author	**Sub-taxa**	**Synonymies**	**Growth form**	**Height in meter**	**number of stems**	**Young branchlets**	**Spines**	**Flowers**	**Leaves**	**Pods**	**Crown**	**Location**
	spirocarpa var *major*		tree	6 - 15	many	pubescent		pale yellow		pubescent	umbrella shaped often flattened	
Andrews 1952	*tortilis*	*A. spirocarpa* Hochst. ex. A. Rich	tree	12		pubescent	straight, long and very small, sharply recurved	Flower-heads white to cream	Pinnae 3-10 pairs; leaflets 7-15 pairs	yellow-brown, pubescent or puberulous, contorted or spirally twisted, slightly constricted between the seeds, circular in cross-section	flat-topped or umbrella shaped	

			Characters used for identification of A. tortilis sub- taxa									
Author	**Sub-taxa**	**Synonymies**	**Growth form**	**Height in meter**	**number of stems**	**Young branchlets**	**Spines**	**Flowers**	**Leaves**	**Pods**	**Crown**	**Location**
	raddiana	*A. tortilis* Hayne	Small to a large tree			glabrous	short, slightly curved and long straight	Peduncle slender	Pinnae 2-5 pairs; leaflet 8-12 pairs	contorted or spirally twisted, flat narrow-linear		
ElAmin 1973	*tortilis*		small tree/ shrub	1 - 4	many						flat	
	spirocarpa		shrubs	4 - 7	2 - 3						flat - spreading	
	raddiana		tree	7 - 12	1						irregular / round	
Täckholm 1974	*tortilis*	*A. spirocarpa* Hochst. ex. A. Rich	tree or shrub	12 - 14		pubescent	long white straight and small recurved dark-tipped	Flowers in head, white to creamy		yellow-brown, twisted, pubescent, up to 115 cm long, 8 mm broad	flat-topped or umbrella shaped	

			Characters used for identification of A. tortilis sub- taxa									
Author	**Sub-taxa**	**Synonymies**	**Growth form**	**Height in meter**	**number of stems**	**Young branchlets**	**Spines**	**Flowers**	**Leaves**	**Pods**	**Crown**	**Location**
	raddiana	*A. tortilis* ssp. *raddiana* (Savi) Brenan)	Tall tree			glabrous		flowers in head		contorted or spirally twisted , flat, glabrous	round irregular crown	
Brenen 1983	*tortilis*		Shrub/ tree	2 - 6		densely or shortly pubescent					flat	Egypt , Sudan
	spirocarpa		tree	2 - 21		pubescent					flat spread	Sudan
	raddiana		tree	1.2 - 10		glabrous to sub-glabrous					round	Egypt , Sudan
ElAmin 1990	*tortilis*		small tree/ shrub	1 - 4	2 - 4						flat - spreading	
	spirocarpa		shrubs	4 - 7	2 - 4						flat - spreading	
	raddiana		tree	7 - 12	1						irregular / round	

			Characters used for identification of A. tortilis sub- taxa									
Author	**Sub-taxa**	**Synonymies**	**Growth form**	**Height in meter**	**number of stems**	**Young branchlets**	**Spines**	**Flowers**	**Leaves**	**Pods**	**Crown**	**Location**
Boulos 1999	*tortilis*		tree		many	pubescent				pubescent	flat-topped	Egypt
	raddiana		tree		1	glabrous				glabrous	irregular	Egypt and Sudan

Paper I

Howaida F. AbdElRahman, Knut Krzywinski. **Environmental effects on morphology of *Acacia tortilis* group in the Red Sea Hills, North-Eastern Sudan and South-Eastern Egypt**.(Accepted: Forest Ecology and Management)

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Environmental effects on morphology of *Acacia tortilis* group in the Red Sea Hills, North-Eastern Sudan and South-Eastern Egypt

Howaida F. AbdElRahman *, Knut Krzywinski

Department of Biology, University of Bergen, Allegaten 41, Post Box 7800, N-5007 Bergen, Norway

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Abstract

Acacia tortilis is a drought-resistant species. Its survival and existence in the arid and semi-arid area of Northern Africa and Arabian Peninsula is due to its ability to endure the harsh condition and therefore, it is generally forms open pure stands or mixed stands in these drylands. Wherever it grows, it plays an important role in human, animal and other plant species lives. Yet the relation between its adaptive features and their relation to the environmental conditions is not studied in a large scale of environmental variation. The main objective of this study is therefore to examine if the immediate environmental factors, other than human utilization, are associated to the expressed morphological variables among the *A. tortilis* sub-taxa. Three subspecies are collectively reported in the study area, Red Sea Hills of Sudan and Egypt. According to different literatures, they are distinguished by features of high plasticity under the variable pressure of human activity and climate. Such features are hair density in some parts of the plant, crown shape and number of stems. In the current study, the result of analysing 520 samples of *A. tortilis* – collected from 25 different localities representing various altitudinal gradients – showed a correlation between the expressed morphological studied and the immediate environment. When data from both regions studied as one data set, elevation, above sea level, was the only significant response variable and higher hair density was associated to plants growing in Sudan. However, when only data from Sudan was studied separately, elevation, hillside and lower part of the catchment were the statistically significant response variables. In the Egyptian data set, the statistically significant response variables were elevation, khor bank and the upper, mid, and lower part of the catchment. An intra-morphological correlation was also shown. These results may suggest that the current *A. tortilis* sub-taxa are ecotype. Genetic and anthropogenic studies are needed to verify this assumption.

Keywords: *Acacia tortilis*; Red Sea Hills; Arid land; Intra-species morphological correlation; Species morphological–environmental correlation

1. Introduction

Trees of African dry lands are not only adapted to high temperature variation and long droughts (Baumer, 1983), extremely low rainfall, high moisture variability and high potential evapotranspiration (Thornthwaite, 1948) but also to intense use by desert dwellers in form of animal browsing, coppice and logging (Krzywinski and Pierce, 2001). *Acacia tortilis* Forssk (Hayne) is one of the most common arboreal species in arid and semi-arid regions across Northern Africa and the Arabian Peninsula (Wickens, 1998; Kassas, 1957; Badi et al., 1989; Manger and Abd ElAti, 1996; Andersen, 1999; Shaltout, 2002). *A. tortilis* is drought resistant, can tolerate strong salinity and seasonal waterlogging and generally forms open forests in pure stands or mixed stands in these drylands. It is also adapted to an environment with little and unreliable rainfall (Ayyad and Ghabbour, 1985) and under these constraints, it is as well adapted to anthropogenic pressures as it is the sole source of animal fodder, firewood and shelter for nomads. Combined pressures and their variation are reflected in its growth forms.

An increasing and pronounced reduction in *A. tortilis* density is reported (Ward and Rohner, 1997; Kennenni, 1990; Andersen, 1999). In the Red Sea Hills of Egypt and Sudan, the combination of climatic and anthropogenic factors, especially charcoal production, is reported to play a key role in this decline in numbers (Alstad, 1991; Christensen, 1998; Krzywinski and Pierce, 2001).

The taxonomy of the subspecies of *A. tortilis* has been considered a puzzle (Brenan, 1957, 1983; Shrestha et al., 2002); http://www.worldagroforestry.org/Sites/TreeDBS/TSSD/synonyms.cfm?sname=Acacia%20tortilis20.05.2005). The morphological features used to separate sub-taxa in the *A. tortilis* complex are largely overlapping and the taxa display

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* Corresponding author. Tel.: +47 55584400; fax: +47 55589667.
E-mail address: howaida.abdelrahman@bio.uib.no (H.F. AbdElRahman).

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high phenotypic plasticity (Brenan, 1957, 1983; El Amin, 1973, 1976, 1990; Täckholm, 1974; Ross, 1979; El Houri, 1982; Kordofani, 1989; Kordofani and Ingrouille, 1992). However, the morphological characters may very well be phenotypic expressions of ecological factors (both biotic and abiotic). For example in arid land, the degree of hair density on certain parts of the plant, regulating evapotranspiration (Stebbins, 1952) and radiation budget (Rozema et al., 1997), and the crown shape, may have been altered by browsing, coppicing and age (Kennenni and Vandermaarel, 1990) and the number of trunks, which can be a result of animal browsing at saplings stage or chopping of trunks for wood.

The rate of speciation is dependent on geographical and ecological variables; therefore, ecological opportunity is the key to speciation and invasion (Levin, 2003). However, since morphological features are highly affected by ecological factors such as climate as well as by human and animal utilization, this may lead to a poor taxonomy when evaluation for speciation is dependent only on characters highly subjected to change. It is characteristic of vegetation patterns in arid landscapes to exhibit a high temporal and spatial variation in growth dependent upon ecological variation in particular soil moisture. The expressed variability in morphology can therefore either be due to environmental or genetic factors. The main objective of the current study is therefore to examine the influence of environmental variables, and spatial variation, on the expressed morphology of *A. tortilis* in the Red Sea Hills in North-Eastern Sudan and South-Eastern Egypt.

2. Materials and method

2.1. Description of the study area

The landscape of the Red Sea Hills can be divided into units defined by climate, geology, and geomorphology (Babiker, 2004). Among these units are the seasonal watercourses (locally are known as khors or wadis), and it is mainly along the flood plains where perennial vegetation occurs. The study area covers two major regions, North-Eastern Sudan (between 18° 40 and 19° 11N latitude and 35° 54 and 37° 5E longitude) and South-Eastern Egypt (between 24° 9N and 25° 5N latitude and 33° 58E and 35° 3E) (Fig. 1). Due to the prevalent climatic conditions, mainly low precipitation and high evapotranspiration, the region can be classified according to the aridity index as arid land (Thornthwaite, 1948). The Egyptian localities fall within the range of hyper arid deserts while the Sudanese localities are in this context less arid (Goldman et al., 1968) (Fig. 2). These climatic conditions in the Red Sea Hills are a product of (1) location within the tropical zone; (2) the presence of the Red Sea as an adjacent body of water, and (3) the Red Sea Hills as an effective physical barrier (El Tom, 1991). The distribution of the available surface and subsurface water resources is governed by the drainage system of the khors/wadis.

The northern (Egyptian) localities therefore experience less frequent summer rain and less influence of orographic rain from the sea than the southern (Sudanese) localities. The high mountains and plateaus in the south act as an effective barrier to climatic influence of air masses from either side of the watershed. This effect is however in places modified by lower altitude mountain passes that created corridors.

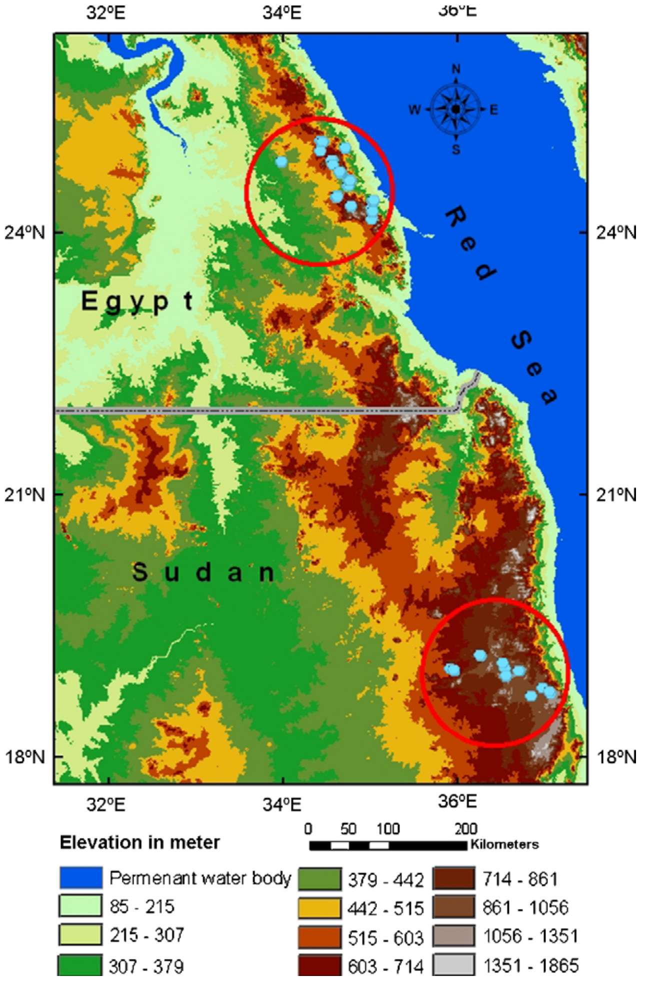

Fig. 1. Map showing the location of the study area, the two main regions are indicated by red circles, based on digital elevation model. Blue dots indicate the various localities studied. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.2. Localities studied and samples collection

In each of the two major regions a number of localities were selected from either upper, middle or lower in the part of the *khor/wadi* (Fig. 3A). A total of 33 localities in the Red Sea Hills in Sudan and Egypt were examined in the period from February to April 2003 (February and March in Egypt and April in Sudan). Of these, 25 were selected for closer study within the frame of reference of this paper to cover the growth of *A. tortilis* along khors/wadis. The elevations in meters above sea level (m.a.s.l.) range from 1079 (Upper Amat in Sudan) to 216 (Wadi Abu Ghusun in Egypt). Sudanese localities were located at higher altitude than those in Egypt. The stated elevations are averages of the elevations of the plant samples in each locality extracted from the free downloaded Digital Elevation Model, source http://seamless.usgs.gov/, using ArcGis version 9.0.1

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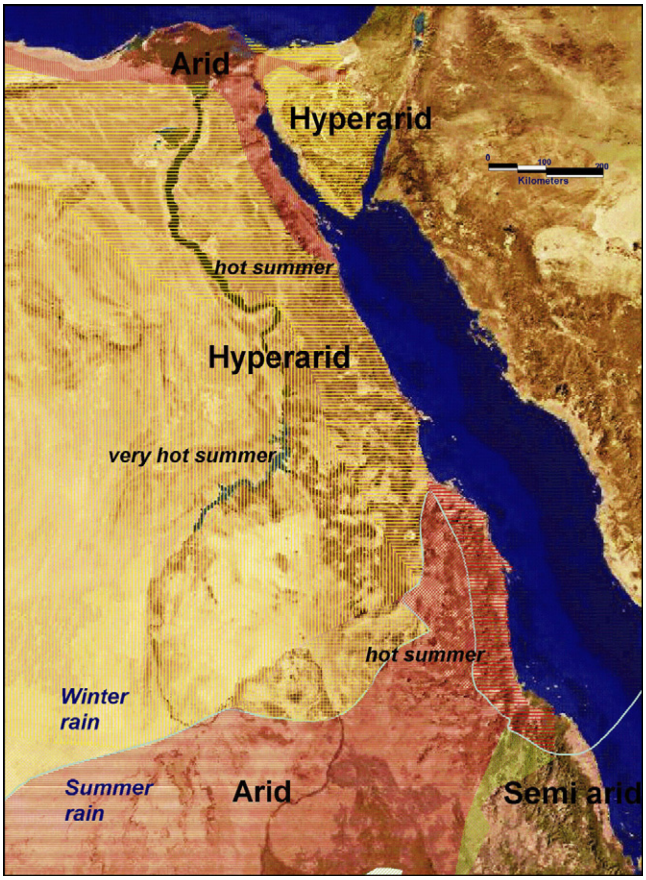

Fig. 2. Climatic map of the study area (Krzywinski and Pierce, 2001).

software. Names, number of samples collected, and elevations of the localities are tabulated in Table 1.

The total numbers of the analysed samples were 520. Samples were collected randomly. Randomness was applied because the various localities were different in their degree of dryness and some trees were without leaves at the time of sampling. The number of collected samples per locality ranged from 4 (Wadi ELJmal 2) to 40 (Wadi Abu Ghusun).

2.3. Variables studied

All variables studied appear in boldface.

Morphological characters of *A. tortilis* were recorded on site and used as the morphological variables for the current statistical analysis. These characters are permanent and not affected by temporal variation and similar to those have been used in relevant literature to identify the sub-taxa of *A. tortilis* and they are the characters used in the flora (Brenan, 1957, 1983; El Amin, 1973, 1976, 1990; Täckholm, 1974; Ross, 1979; El Houri, 1982; Kordofani, 1989; Kordofani and Ingrouille, 1991, 1992). Reproductive organs are not included in this study because they were hardly found in all samples. With the exception of **height**, which is measured in meters from the base of the stem at the ground level to the top of the crown, all other variables are presence/absence variables coded as 1 if the variable is present or 0 if the variable is absent from the plant studied. These variables are (Fig. 4): growth form (**tree/shrub**); number of stems from ground level (**stem = 1/stem = 2–5/stem > 5**); distribution of hairs when seen by the naked eye (**no hair/only in the petiole/young branchlets densely pubescent**); the shape of the crown (**round/flat/spread/undefined**); spine shape (**spine = straight/spine = mixed**, i.e., both straight and curved). Hair density and the height of the plant, to some extent, are the only variables that are associated with the plant adaptation to the natural environment (Stebbins, 1952), while the remaining variables are more subjected to change due to human activities or animal browsing.

Three major response variables, representing the environmental factors, were recorded; two of them were sub divided into three sub-variables and were coded 1/0 for the presence/absence: namely, location of the plant within its locality (Fig. 3B) (**mid – khor/khor bank/hill side** reflected distance from main drainage line) and position in khor/wadi (Fig. 3A) (**upper run/mid run/lower run** reflecting size of catchments). They represent the cross-sectional and longitudinal distribution of the plants with respect to the water distribution of the khor/wadi, respectively. The third variable is the **elevation** measured in m.a.s.l. of each plant. The elevation is not only connected to catchments size it also reflects that at high elevations there is more frequent light showers, dewfall and day/night differences of moist air. At the same time, there are less frequent, less high intensity torrents and floods.

To establish if the revealed correlation between the studied morphological variables concurs the previously described features (El Amin, 1973, 1990; Brenan, 1983) to differentiate between the various taxa of *A. tortilis*, the expected taxa was then classified accordingly and treated as supplementary variables. These sub-taxa were spp. **raddiana** (one stem, round to irregular crown, glabrous to sub glabrous), spp. **tortilis** (multi stem, flat crown, densely or shortly pubescent), spp. **spirocarpa** (2–5 stem, flat-spreading crown, pubescent), and **unclassified** if the characters are overlapping.

2.4. Statistical analysis

Since the dataset contains categorical variable contingency table was used to organise the dataset (Gotelli and Ellison, 2004). A series of analyses of the contingency tables cross-tabulating key variables was performed using the licensed XLSTAT^{TM} (www.xlstat.com). This procedure tests whether the variables cross-tabulated are statistically independent of one another and quantifies the degree to which they are associated. In all cases alpha was set to 0.05.

In view of the fact that the two major regions studied are different in their aridity, the statistical analysis is designed to study the influence of the immediate habitat among and within the two regions. That is, data from both regions were studied as one set of data, and afterwards data from each region were treated separately. Since the present data set examine the behaviour of more than one dependent variable, ordination techniques were applied to determine the relation between the

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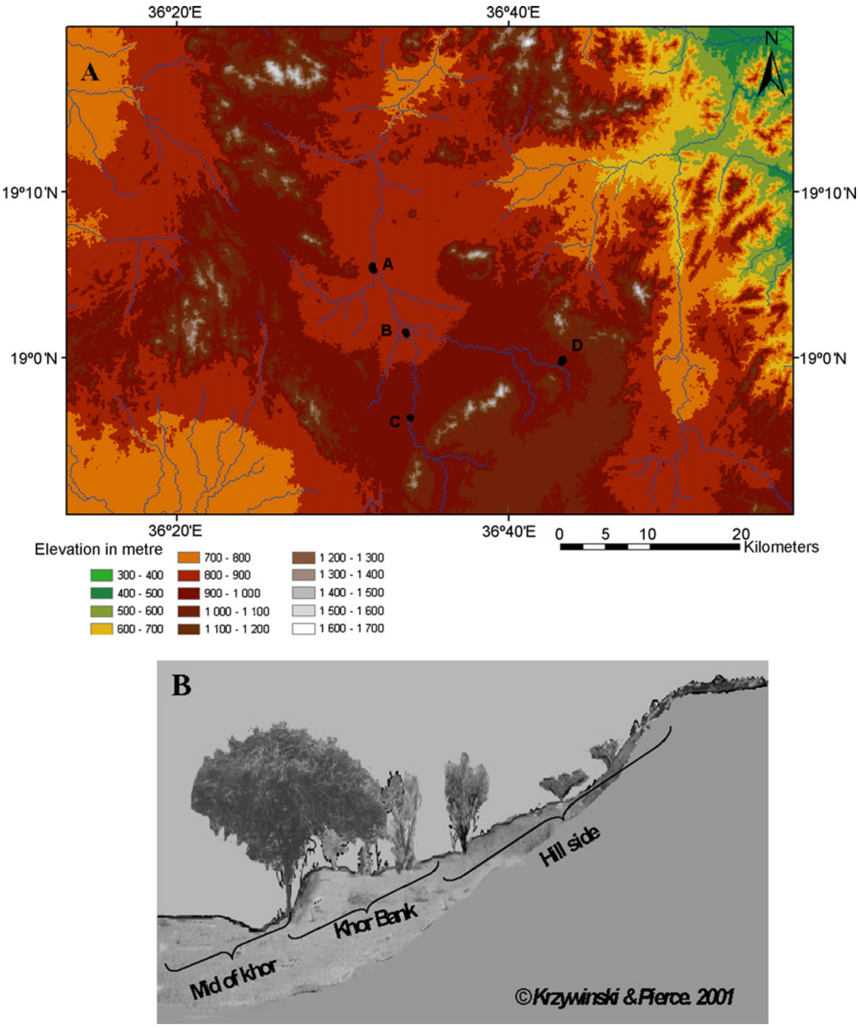

Fig. 3. Longitudinal and cross-section catchment (khor/wadi). (A) Map demonstrating the longitudinal structure of the catchment. In this illustration some of the Sudan's localities are shown to represent what is meant by the upper (c and d), mid (b) or lower (d) locality in the part of the catchment studied. Blue line indicates khor/wadi. (B) Cross-section sketch of the plant distribution with respect to the catchment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

environmental variables and the morphological variables studied, using CANOCO for Windows version 4.5, and the graphical representations were done using CanoDraw for Windows version 4.0 (ter Braak and Šmilauer, 1998).

To determine which of the ordination method, the linear or the unimodal technique, was suitable for the available data, decided by the gradient length when applying Detrended Correspondence Analysis (DCA) (Leps and Smilauer, 2003). The gradient length measures the beta diversity in community composition along the individual independent gradients. Therefore, DCA is a reliable method to perform that because it provides non-linear scaling of the ordination axes in units of mean standard deviation of species turnover (Okland, 1999). It also prevents any systematic relationship to the ordination axes. The largest value was 2.204 S.D. This value gives the freedom of using either the unimodal or the linear techniques. The indirect techniques of both models were applied and the model that showed higher explanation of the morphological variability using the environmental variables was followed.

The unimodal technique, Correspondence Analysis, CA, and the linear technique, Principal Component Analysis, PCA, were applied to study the variability in the morphological characters with respect to the environmental variables (Table 2). To test which of the methods give more strength between the morphological variables and the environmental variables, the equation (r_*^2 the eigenvalues) of the strong axes was applied; where r is the strength of the relation between the morphological variables and the environmental variables for that axes (ter Braak and Šmilauer, 1998). It was found out to be 0.068 for the linear model and 0.044 for the unimodal. As well the total variance was higher in the linear method than the unimodal (0.107 and 0.099, respectively). Therefore, the linear technique explained better (by, expressed in percent, 6.8% and 10.7%) the morphological variables by environmental variables. Hence, further analysis was continued following the linear techniques. To quantify for the effect of the environmental variables on the expressed morphological variables, a constrained technique, Redundancy Analysis (RDA) was then

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Table 1
Studied localities; name and elevation, and number of collected samples per locality of the studied regions

Major region	Locality name	Elevation average (m.a.s.l.)	Collected samples
Egypt	Lower Wadi Hulus	439	22
	Wadi ElJimal 1	286	6
	Wadi ElJimal 2	285	4
	Wadi Nuqrus	304	29
	Wadi Sukkari	300	32
	Wadi Hanjaliyya	707	31
	Wadi Sartut 2	347	32
	Wadi Abu Ghusun	216	40
	Wadi Hulayfi	513	28
	Upper Wadi Nuqrus	600	35
	Mid Wadi Nuqrus	421	27
	West Wadi Hanjaliyya	565	27
	Wadi Muylhie	358	24
Sudan	Upper Beramio	1052	10
	Lower Beramio	920	10
	Upper Amat	1079	20
	Lower Amat	1018	20
	Upper Hilayet	973	27
	Nubahweeb	883	17
	Upper Biramfi	926	13
	Dybadwat	852	11
	Lakatyeb	816	25
	Upper Sarara Agwampt	642	10
	Lower Sarara Agwampt	611	12
	Mid Sarara Agwampt	631	8

followed. Program default settings were followed with the exception of using the log transformation in the transformation of the species step so as to prevent taking the log of the zero values.

Monte Carlo permutation (499) test was applied to study the significance of the environmental variables. The two main regions were used as blocks to restrict the permutation test. The significance level p-value ≤ 0.05 was used to indicate which of the environmental variables that contribute most to the variation in the morphology within the studied samples.

The above-mentioned steps for the statistical analysis were performed on the entire data set of both regions. Steps after the decision of which model to be followed were repeated for the data set of each region separately.

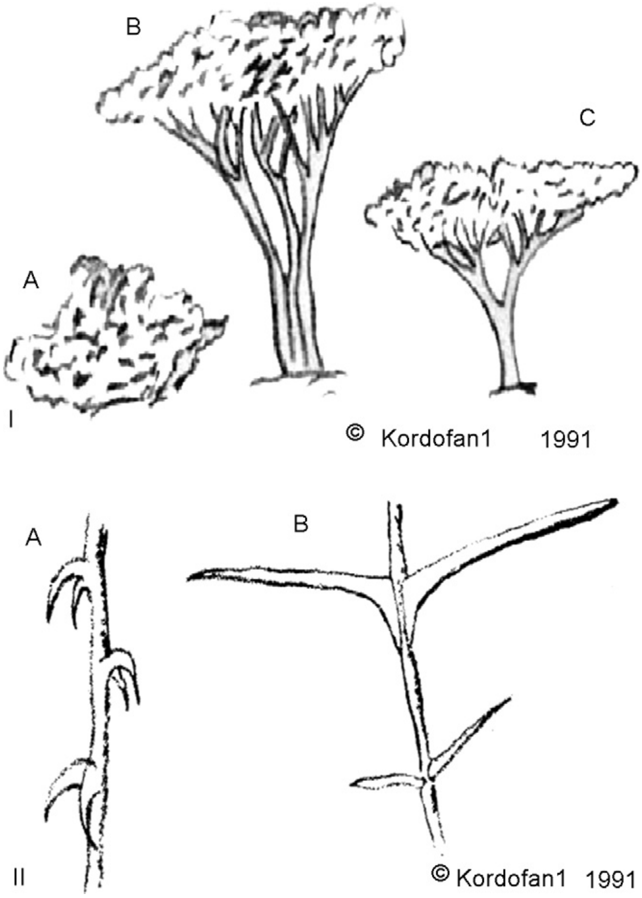

Fig. 4. Illustration of the morphological variables. (I) Growth form (A) Shrub; (B and C) Tree. Number of stems (B) three stems; (C) one stem. (II) Spine shape; (A) curve and (B) straight.

3. Results and discussion

3.1. Cross-tabulating key variables

As regards the morphological variables, **hairs** and **spines** were not distributed evenly among all the acacias, nor did they appear on all parts of the ones where they were found. Where they did occur, however, they were statistically linked (Chi-sq = 7.24, $p = 0.027$) and positively associated (Pearson's phi = 0.118).

When, however, **spines** were cross-tabulated with **taxa**, they were randomly distributed among the taxa (Chi-sq = 3.128,

Table 2
Summary of the CA and PCA to study the variations on the morphological features (values in bold are used in discussion below)

	CA					PCA				
	1^{a}	2^{a}	3^{a}	4^{a}	Total inertia	1^{a}	2^{a}	3^{a}	4^{a}	Total variance
Eigenvalues	0.264	0.198	0.157	0.135	1.259	0.328	0.143	0.112	0.086	1.000
Morphological–environment correlations	0.408	0.271	0.159	0.271		0.458	0.174	0.296	0.321	
Cumulative percentage variance										
of morphological data	21.0	**36.7**	49.2	59.9		32.8	**47.1**	58.3	66.9	
of morphological–environment relation	44.2	**58.9**	62.9	72.9		64.1	**68.2**	77.4	85.6	
Sum of all canonical eigenvalues			**0.009**					**0.107**		

[a] Axes.

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Table 3
Summary of the PCA, to study the variation on the morphological features (values in bold are used in discussion below)

	Axes				Total variance
	1	2	3	4	
Eigenvalues	0.328	0.143	0.112	0.086	1.000
Morphological–environment correlations	0.458	0.174	0.296	0.321	
Cumulative percentage variance					
of morphological data	32.8	**47.1**	58.3	66.9	
of morphological–environment relation	64.1	**68.2**	77.4	85.6	
Sum of all canonical eigenvalues					**0.107**

$p = 0.372$) and positively associated (Pearson's phi = 0.078), whereas **hairs** was clearly linked to **taxa** (Chi-sq = 100.99, $p < 0.0001$) and markedly positively associated (Pearson's phi = 0.440).

When **hairs** and **spines** were cross-tabulated with the variable **region**, i.e. Sudan and Egypt, they were statistically dependent, both being clearly linked to region ($p < 0.0001$ for Chi-sq in both cases), with **hairs** strongly positively associated (Pearson's phi = 0.749) and **spines** negatively (Pearson's phi = −0.217). Of acacias in Egypt 31.8% had **no hairs** and 60.2% had **hairs only on petiole**, whereas 81% in Sudan had **hairs on all branchlets**. As for **spines**, acacias in both Egypt and Sudan were predominantly **straight** (81.3% and 96.7%, respectively), but very disproportionately so as regards **mix** (91.3% and 8.7%, respectively).

Finally, the relation between **taxa** and **region** is one of significant dependency (Chi-sq = 73.09, $p < 0.0001$) with a clearly positive association (Pearson's phi = 0.375). Here, it is the predominance of spp. *raddiana* in Egypt (82% of all the spp. *raddiana* recorded and 34.2% of the total proportion of all the acacias classified in the entire dataset) that characterizes the data.

The shape of the **crowns** was clearly linked to the number of **stems** (Chi-sq = 113.95, $p < 0.0001$) and positively associated (Pearson's phi = 0.468).

3.2. Decide which technique to be applied

For the reasons mentioned above (Section 2.4), the linear techniques were chosen to determine the relation between the morphological variables and the environmental variables recorded. In Table 2, the first two axes explained 36.7% and 47.1% variability in the morphological variables, whereas, by the morphological–environmental data it is 58.9% and 68.2%, respectively. These results strengthen the arguments for linear method choice to be followed for the rest of the analysis. In addition, the variation in the amount explained suggested that the response variables did contribute to the variation in the morphological variables.

3.3. Linear techniques: PCA

From Table 3, the environmental variables used to explain the variability in the morphological variables explained only 10.7% (expressed here in percent) of the total variability in the morphological data.

3.4. Direct gradient analysis of the linear models: RDA

By applying RDA, the analysis is constrained by the environmental variables. As shown in Table 4 below, the first two axes explained 93.7% of the morphological variables as explained by the environmental variables.

Egypt and Sudan are located in regions of different degree of aridity (Goldman et al., 1968). To study if that factor can account for the different morphological variables that the plants studied expresses, the two major localities were used as covariables. The result is summarized in Table 5. The amount of variation that can be explained with the covariables is $(100*[1 - 0.875] = 0.125)$, that is 12.5%. The sum of all canonical eigenvalues measures the amount of variation in the morphology that can be explained by the environmental

Table 4
Summary of the direct gradient analysis, RDA

	Axes				Total variance
	1	2	3	4	
Eigenvalues	0.090	0.011	0.003	0.002	1.000
Morphological–environment correlations	0.575	0.327	0.168	0.147	
Cumulative percentage variance					
of morphological data	9.0	10.0	10.4	10.6	
of morphological–environment relation	83.8	93.7	96.9	98.8	
Sum of all canonical eigenvalues					0.107

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Table 5
Summary of RDA including the major localities as covariables

	Axes				Total variance
	1	2	3	4	
Eigenvalues	0.016	0.009	0.006	0.002	1.000
Morphological–environment correlations	0.375	0.272	0.170	0.154	
Cumulative percentage variance					
of morphological data	1.8	2.9	3.5	3.7	
of morphological–environment relation	46.3	73.2	89.6	95.1	
Sum of all eigenvalues					0.875
Sum of all canonical eigenvalues					0.034

Table 6
Summary of the RDA-Monte Carlo permutation analysis for the statistically significant environmental variables

	Axes				Total variance
	1	2	3	4	
Eigenvalues	0.012	0.259	0.128	0.106	1.000
Morphological–environment correlations	0.317	0.000	0.000	0.000	
Cumulative percentage variance					
of morphological data	1.3	31.0	45.6	57.7	
of morphological–environment relation	100.0	0.0	0.0	0.0	
Sum of all eigenvalues					0.875
Sum of all canonical eigenvalues					0.012

variables. This amount was found to be lower (Tables 4 and 5) when the variation among the morphological variables was restricted to the two regions. This indicates that certain (may be all) of the studied environmental variables accounted for the variation in the morphological variables.

To evaluate which of the studied environmental variable(s) did contribute to the variability in the morphological variables, the Monte Carlo Permutation test was applied. At the significance level of p-value ≤ 0.05, only the elevation, above sea level, showed significance (Table 6).

This indicates that the elevation, as the only significant response variables, explained only 1.2% out of 10.7% (expressed here as percent) of the total variability of the morphological variables. To illustrate the correlation between the morphological variables and the elevation, CanoDraw was used to produce a plot which shows this relation (Fig. 5).

The morphological variable expected to be least affected by the anthropogenic activities is the pattern of the indumentums of the leaf (Baleela and Kordofani, 1995). The subspecies are separated by differences in the pubescence of the branchlets (Brenan, 1983). From Fig. 5, it can be predicted that the higher the elevation (above sea level) of the locality the less hair density will be observed among the *A. tortilis* species. But hair in the leaves increases boundary layer during the process of transpiration to regulate the loss of water according to the environment at which the plant is growing, and as well regulate the plant temperature by controlling the leaf energy budget in a form of reflecting the light. Our results agree with these assumptions. From Table 1, the most elevated locality is Upper Amat. The climatic condition of this locality is somewhat different from the rest of the localities due to its nearness to the mist oasis of Erkwit (Kassas, 1956). The air humidity is on one side higher in this locality but on the other

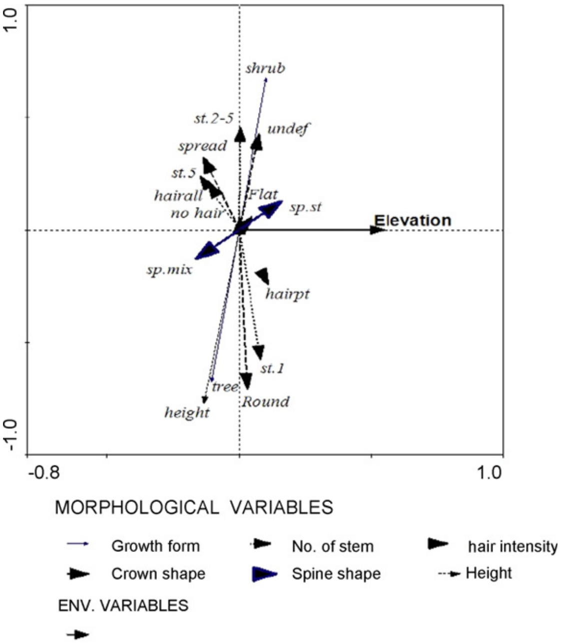

Fig. 5. Graphical representation of the relation between the elevation and the studied morphological variables (st. = no. of stems; hairpt = hair in petiole, hairall = hair in branchlet; undef = undefined crown shape; sp.mix = spines are mixed, sp. st = spines are straight).

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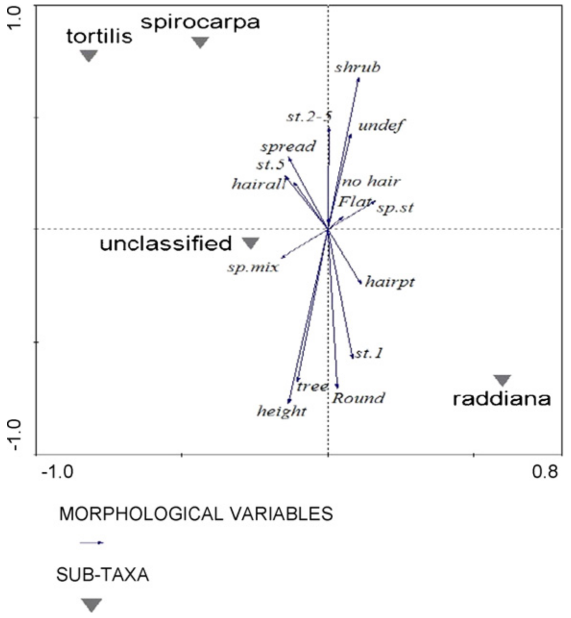

Fig. 6. Relation between the studied morphological variables and the expected taxa. Abbreviations used in this figure are the same as in Fig. 5 above.

the khor drains most of the water from the mist oasis and therefore one may expect that the soil moisture is also very high.The height is negatively correlated with the first ordination axes whereas the elevation is positively correlated (Fig. 5). According to Kennenni and Vandermaarel (1990), the higher the elevation, the better moist air condition but less subsurface soil moisture. The negative correlation can be attributed to the fact that at the lower part of the catchment, where water drains from larger catchment (Fig. 3A) during rainy periods, the amount of soil moisture is in general relatively higher than in elevated areas.

In spite of the fact that the morphological features used to differentiate between the sub-taxa of *A. tortilis* overlap, the results (Fig. 6) concur with what was described in the previous literature (El Amin, 1973, 1990; Brenan, 1983). From the previous literature, spp. *raddiana* is distinct from both spp. *spirocarpa* and *tortilis*, which share most of their common features. This is agrees also with the present results as it can be seen from Fig. 6 that the distance between spp. *tortilis* and spp. *spirocarpa* is shorter than each to spp. *raddiana*. The round crown is the main feature that distinguishes spp. *raddiana*. From Fig. 6, the round crown is more correlated to the spp. *raddiana* than the other crown shapes.

As elevation was the only response variable that moderately explained the difference in the morphology of *A. tortilis* in Sudan and Egypt, variation in the morphology was also observed among *A. tortilis* growing in the different localities within the same region, i.e., Sudan or Egypt. To study localized regional variation, the same statistical analysis applied above to examine the effect of environmental variables, was repeated for each region separately. This was performed by running the analysis two more times but each time excluding samples from the other region.

Considering the samples collected from Sudan, the statistically significant environmental variables were found to be elevation of the locality above sea level, the plant being growing in the hillside and the locality studied being at the lower part of the catchment. Collectively, they explained 9.5% of the total expressed morphological features. In Fig. 7, the correlation between the statistically significant environmental variables and the morphological variables is exemplified. As mentioned above, the least affected morphological variables by anthropogenic activities are the hair density and the height of the plant. In connection to this and from Fig. 7, it was noticed that the elevation was negatively associated with the higher density of the hair and the height of the plant. This can be attributed to the fact that at high elevations differences between the day and night temperature forming dewfalls which will condense at the surface of the plant (e.g. spines, leaves, and hairs). And it might be due to this assumption the plants growing on the localities studied do response and modifies their morphological variables accordingly.

When the samples collected from Egypt were analysed, the statistically significant environmental variables were the elevation, khor bank, and the lower and the upper parts of the catchment. Collectively they explained only 5% of the total variability in the morphology of the *A. tortilis* studied. The association between these environmental variables and the morphological variables can be demonstrated in Fig. 8. Still the elevation and the higher hair density show negative correlation. This is shown by their opposite relation to the first ordination axes. The soil moisture is relatively higher at the lower part of the catchment, therefore the plant need lesser hair to regulate its evapotranspiration.

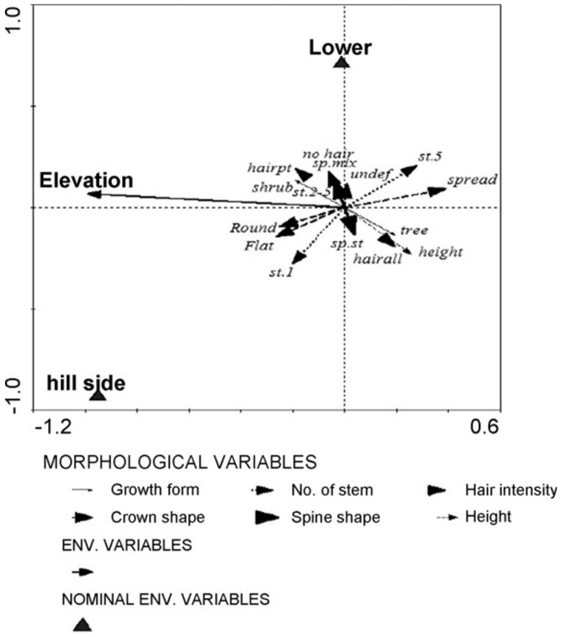

Fig. 7. Graphical representation of the relation between the statistically significant environmental variables and the morphological variable of samples collected from Sudan. Abbreviations used in this figure are the same as in Fig. 5 above.

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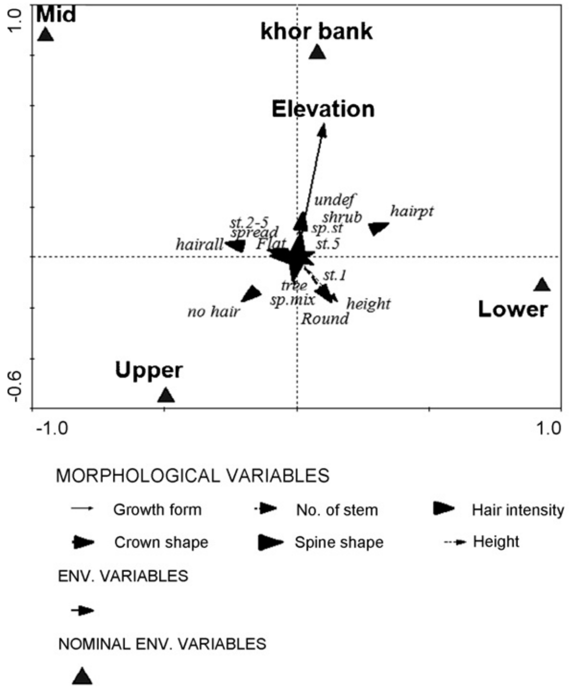

Fig. 8. Graphical representation of the relation between the statistically significant environmental variables and the morphological variable of samples collected from Egypt. Abbreviations used in this figure are the same as in Fig. 5 above.

4. Conclusion

This work is the first of its type to study the relation between the morphological variables and the environmental variables of *A. tortilis* along aridity gradient of the Red Sea Hills. Result showed that the environmental variables did contribute to the expressed morphological variables expressed by the *A. tortilis* studied. In spite the contribution was generally low but this can be an indication that along climatic gradient the plants are forced to modify their characters so as to be able to adapt to the new environment. In arid lands, such rate of evolution is speeded up (Stebbins, 1952). Results also showed that elevation is the main response variable that indicated significance to the expressed morphological variables of the entire plant studied. Elevation is negatively correlated to both increased amount of hair density and height of the plant. The position along the khor/wadi (Fig. 3) is related to soil moisture gradient (Kennenni and Vandermaarel, 1990). Availability of soil-moisture is the vital factor for sustainable life in arid lands. The two regions studied vary in their degree of aridity (Goldman et al., 1968) and different environmental factors can affect the growth of the plant. The morphology of the plant collected from Sudan was explained by 9.5% of the environmental variables. These environmental variables were elevation, hillside and lower part of the catchment. On the other hand, the samples collected from Egypt, the expressed morphology was explained by only 5%. These environmental variables are the elevation, khor bank and the longitudinal structure of the khor/wadi. All these environmental variables that share their relation to moisture conditions showed different degree of correlation to the density of the hair in the branchlets. The presence of hair, as air moisture/evapotranspiration regulator, as well as energy budget regulator, in the various parts of the plants reflects adaptation of the plant to arid environment. Therefore, the current results the local environmental variations play an important role on shaping the plant morphology. These results are consistent with the view that if conservation of species diversity is targeted, then environmental factors should be considered with special focus on local variations. This consideration can help in establishing forest management practices in arid land. For example, in Egypt plans were set to establish a regeneration program and to develop technique to protect the species. Currently, the application of such program and technique is under progress (Hatab, 2003). On the other hand, in Sudan no comprehensive programs were set to regenerate or protect the species (Christensen, 1998). In spite of the low percent that the environmental variables can explain in the three cases studied (i.e. the two regions as entire and each one separately) results from this study can act as a good start for further analysis of *A. tortilis* along more variant aridity gradient; such as a study of the effects of human use and management on the morphology of *A. tortilis*. The morphological features used to separate the sub-taxa of *A. tortilis* (El Amin, 1973, 1990; Brenan, 1983) agree with the results from this study (Fig. 6). However, whether the current sub-taxa of the *A. tortilis* are just ecotypes or different species or subspecies should await genetic verification using the DNA pattern of the studied plants. Such studies are needed to understand the autoecology of the species, update the taxonomy of the species, improve the understanding of the species dynamics, better understanding of the species environmental planning and management, assessing the impact of the environment in the species distribution, make better policy for managing regeneration programs, if needed, since the species is facing high rate of mortality. Similar studies can be performed on species that are faced with extinction.

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Paper II

Howaida F AbdElRahman, Knut Krzywinski, Richard H Pierce. **Heterogeneity in group clustering of *Acacia tortilis* of the Red Sea Hills in Sudan and Egypt using morphological and molecular data**. (Revision with reviewers BMC Ecology).

Heterogeneity in group clustering of *Acacia tortilis* of the Red Sea Hills in Sudan and Egypt using morphological and molecular data.

Howaida F AbdElRahman[1]*, Knut Krzywinski[1], Richard H Pierce[2]

[1]Department of Biology, [2]Department of Greek, Latin and Egyptology, University of Bergen, 5007 Bergen, Norway.

Emails: Howaida F AbdElRahman* - howaida.abdelrahman@bio.uib.no; Knut Krzywinski - knut.krzywinski@bio.uib.no; Richard H Pierce - richard.pierce@krr.uib.no

*Corresponding author

Abstract

Background: *Acacia tortilis* is among the few plant species able to survive the harsh environmental conditions of deserts in Northern Africa and the Arabian Peninsula. It is the fundamental source of animal fodder and shelter for nomads. Yet, in spite of its importance, the taxonomy of its sub-taxa is still open to question. This is because the taxonomic characters used to distinguish them are mainly morphological and on closer inspection subject to climatic as well as anthropogenic influences. The main objective of this study is to assess taxonomic relationships in the *A. tortilis* complex in the Red Sea hills in Sudan and Egypt, using both morphological and molecular data.

Results: The Random Amplified Polymorphic DNA (RAPD) method was used to study DNA variation among 106 samples of *A. tortilis*. To compare the morphological and molecular data XLSTAT Addinsoft™ software was employed for cluster analysis. None of the methods applied showed consistency between class members using the two sets of data (DNA and morphology). Preliminary results obtained show a high level of genetic polymorphism among the samples studied. Plants with different morphological features show the same DNA fragments with regard to some primers.

Conclusion: The study concludes that, when only morphological characters are used, serious doubt about sub-divisions in *Acacia tortilis* arises. The morphological characters are still useful as ecological indicators, but DNA studies are needed to verify any true taxonomic subdivisions in the *A. tortilis* complex. The current result,

therefore, addresses the need to perform a population genetic study of these samples so as to determine what factors are causing the genetic variation on the current samples studied and to study the degree of significance of their genetic variations.

Background

The highly drought tolerant desert acacias in north Africa are of great importance for desert-dwellers in that region [1]. In African drylands, desert acacias, being deeply rooted, are well adapted to extremely low rainfall, long droughts [2], high moisture variability and potential evapo-transpiration [3]. Observations in the field made over many years have raised doubts in the minds of the authors as to the taxonomic status of the acacias growing in the Red Sea Hills (RSH) in Egypt and Sudan. When the standard morphological characters used to classify *A. tortilis*, *A. spirocarpa*, and *A. raddiana*, are applied consistently in the field, it proves virtually impossible in practice to identify a large proportion of the trees observed. The problem resides partly in the characters themselves, some of which can be shown to reflect the effects of environment, intense utilization by desert dwellers, and animal browsing rather than the direct effects of their genetic makeup [4]. These factors can account for the variation in the morphological patterns that these desert plants express, but in most cases in the past morphological patterns have been the basis for plant taxonomy, especially at lower levels, e.g. the species level. This has also been true for the *Acacia tortilis* Forssk (Hayne), which includes the most common arboreal species in arid, semi-arid and even hyperarid regions across Northern Africa and the Arabian peninsula [5-9]. Although taxonomists have tended to agree that this complex is represented by three "sub-species" in the eastern desert and RSH of Egypt and Sudan, viz. spp. *tortilis*, spp. *raddiana* and spp. *spirocarpa* [10], the morphological characters used to establish this taxonomy have been applied inconsistently. Those used to differentiate between spp. *tortilis* and *spirocarpa* overlap, while those used to distinguish spp. *raddiana* are distinct [10-12]. The morphological characters used to distinguish these three sub-species are displayed in Table 1. Moreover, although there is a considerable environmental continuity between Egypt and Sudan, spp. *spirocarpa* has been reported only in Sudan but not in Egypt, whereas the other two sub-species are common in both regions. It was to clarify our doubts about this taxonomy that the research presented below was initiated. Environmental differences may also account for differences in community structure. In the study area, specimens of the *A. tortilis* complex are found growing along *khor*s (or *wadi*s; in this study *khor* and *wadi* will be used interchangeably, in both cases referring to normally dry channels which receive run-off water during rainy seasons) in the RSH in Sudan and Egypt. In the khors vegetation varies from one zone to another and in accordance with the size of the catchment area, the slope and the extent of the flood plain, and the depth and texture of the bottom deposits [13]. The main objective of this study is to find out whether the current characters that are used to distinguish between the "sub-species" of *Acacia tortilis*, growing on the RSH of Sudan and Egypt, are diagnostic when they are compared using genetic data.

Table 1
Morphological characters used to distinguish sub-taxa of the *Acacia tortilis* complex in the region studied. Empty cells mean the author did not apply the character mentioned.

Sub-taxa	**Brenen 1983**			**ElAmin 1990**			**ElAmin 1973**		
	tortilis	*spirocarpa*	*raddiana*	*tortilis*	*spirocarpa*	*raddiana*	*tortilis*	*spirocarpa*	*raddiana*
Growth form	Shrub/ tree	tree	tree	small tree/ shrub	Shrubs	tree	small tree/ shrub	shrubs	tree
Height in meters	2-6	2 - 21	1.2 - 10	1-4	4-7	7-12	1-4	4-7	7-12
Stem number				2 - 4	2 – 4	1	many	2-3	1
Young branchlet	densely or shortly pubescent	pubescent	glabrous to sub-glabrous						
Crown	flat	flat spread	round	flat-spreading	flat-spreading	irregular / round	flat	flat-spreading	irregular / round
Location	Egypt, Sudan	Sudan	Egypt, Sudan						

Description of the study area:

The two sets of localities (twenty in all) sampled for this study lie in the RSH at what can be seen as the northern and southern ends of a 680 km long gradient of gross water availability that extends from South-Eastern Egypt (between 24° 9 N and 25° 5 N latitude and 33° 58 E and 35° 3 E) to North-Eastern Sudan (between 18° 40 and 19° 11 N latitude and 35° 54 and 37° 5 E longitude) (Figure 1). The landscape of this vast region can be analyzed into units defined by climate, geology, and geomorphology [14]. Prominent among these units are the khors where the samples used in this study were collected. Due to the prevalent climatic conditions, mainly low precipitation and high evapotranspiration, the region as a whole can be classified according to the aridity index as arid land [3]. The Egyptian localities fall within the range of hyperarid deserts while the Sudanese localities are, in this context, less arid [15]. These climatic conditions are a product of (1) location within the tropical zone; (2) the presence of the Red Sea as an adjacent body of water, and (3) the role of the RSH as an effective physical barrier [16]. The distribution of the available surface and subsurface water resources is governed by the drainage system of the khors.

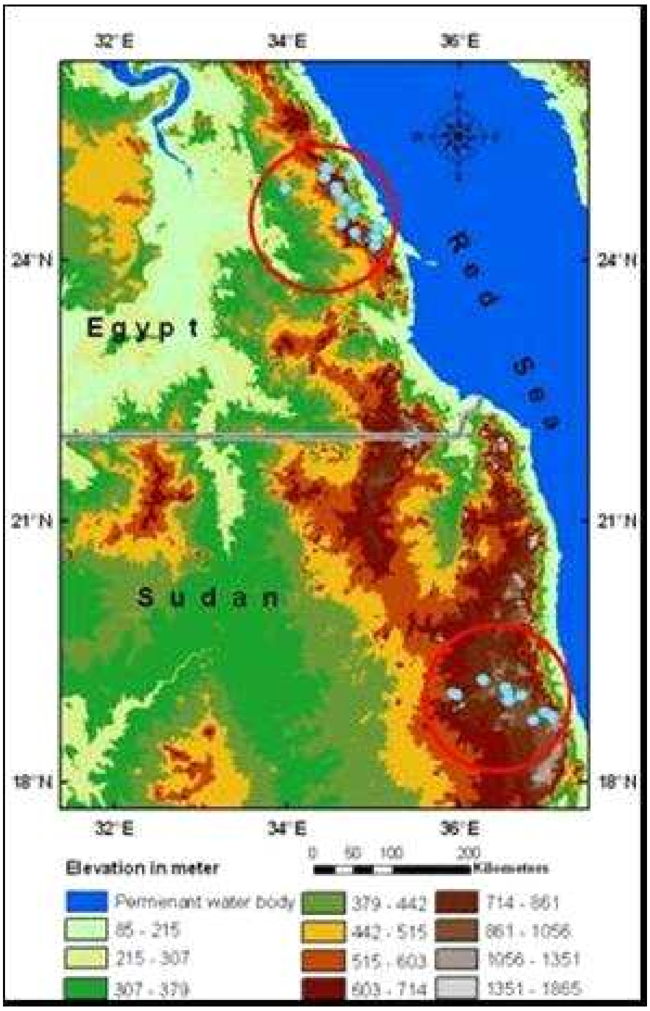


Figure 1
Study area. Small blue circles within larger red circles represent the geographical locations of the studied localities at the RSH, South East Egypt and North East Sudan based on a DEM.

The northern (Egyptian) localities experience less frequent summer rain and less influence from orographic rain from the sea than the southern (Sudanese) localities. The high mountains and plateaus in the south act as an effective barrier to the climatic influence of air masses from either side of the watershed. This effect is, however, modified in places by lower altitude mountain passes that create corridors.

Results and Discussion

Chi-square Automatic Interaction Detection (CHAID)

As can be seen from the introduction and Table 1, the number and adequacy of morphological characters that have been specified for field workers to distinguish eventual **subtaxa** of *A. tortilis* in the RSH are open to question. In practice a researcher in the field may mobilise additional observations such as the on-site distribution of trees or general past experience to make an identification; but if only the characters formally specified in Table 1 were rigorously applied, there would probably be many trees whose taxonomy would remain in doubt. To test this expectation one of our team attempted to classify each of the 520 *A. tortilis* recorded in the field according to the established characters. The result was 217 trees classified as *raddiana*, 138 as *spirocarpa*, and 45 as *tortilis*, while 120 remained unclassified. This classification was added as a supplementary column to MORPHNOM520 and this table then processed in CHAID using our nominal taxonomic classification as the dependent variable and the nominal morphological characters as the independent variables. The CHAID application produced a classification very close to ours. This is summarized in the following confusion matrix (Table 2), showing the prior and posterior classifications and the changes that took place. The agreement between the two classifications was found to be 95.77%.

When the same procedure was followed for the subset MORPHNOM106 (i.e. 106 samples selected from the above mentioned 520 samples), there was a slightly improved correspondence between the prior and posterior classifications (Table 3). The agreement between the two classifications was found to be 96.23%. The same procedure was then repeated, replacing the nominal morphological characters with the corresponding bands of DNA106 as the independent variables. This time 31 taxa were reclassified (Table 4), and the agreement between the two classifications was found to be 80.19%.

Table 2

CHAID MORPHNOM520. Matrix created by CHAID showing the prior and posterior morphological classification of 520 samples and the changes that took place when both classifications were compared.

from \ to	raddiana	spirocarpa	tortilis	unclassified	Total	% correct
raddiana	217	0	0	0	217	100.00%
spirocarpa	0	136	0	2	138	98.55%
tortilis	0	3	42	0	45	93.33%
unclassified	7	9	1	103	120	85.83%
Total	224	148	43	105	520	95.77%

Table 3

CHAID MORPHNOM106. Matrix created by CHAID showing the prior and posterior morphological classification of 106 samples and the changes that took place when both classifications were compared.

from \ to	raddiana	spirocarpa	Tortilis	unclassified	Total	% correct
raddiana	34	0	0	1	35	97.14%
spirocarpa	0	31	0	0	31	100.00%
tortilis	0	0	13	0	13	100.00%
unclassified	0	3	0	24	27	88.89%
Total	34	34	13	25	106	96.23%

Table 4

CHAID DNA106. Matrix created by CHAID showing the prior and posterior molecular classification of 106 samples and the changes that took place when both classifications were compared.

from \ to	raddianna	spirocarpa	Tortilis	unclassified	Total	% correct
raddianna	30	1	1	3	35	85.71%
spirocarpa	0	30	1	0	31	96.77%
tortilis	0	3	9	1	13	69.23%
unclassified	5	5	1	16	27	59.26%
Total	35	39	12	20	106	80.19%

Table 5

CHAID DNA106 and MORPHNOM106. Relation between DNA106 and MORPHNOM106 data. Columns: DNA classes; rows: morphological classes

from \ to	Class1	Class2	Class3	Total	% correct
Class1	91	0	0	91	100.00%
Class2	8	0	0	8	0.00%
Class3	7	0	0	7	0.00%
Total	106	0	0	106	85.85%

In spite of the high percentage of the agreements between what the authors managed to classify in the field and what the program managed to classify of the same samples (Table 2,3 &4), these proved the uncertainty that a researcher would face trying to use morphological characters to classify samples to sub-species levels in the field.

A further attempt to explore the relation between the DNA106 and MORPHNOM106 data involved replacing as the dependent variable the classification done by our team with one of the classifications generated using Agglomerative Hierarchical Clustering, AHC, (UPGMA: Unweighted Pair Group Method with Arithmetic Mean), using Dice's Index and truncating for three classes). This produced the following confusion matrix for the resultant classes (Table 5), and the agreement between the two classifications was found to be 85.85%. In this case all the samples were placed in class 1.

During both procedures to complete the classifications CHAID merged some values into new, self-contradictory ones such as (*petiole*; *none*) and (*none*; *dense*) for **Hairs** and (*round*; *flat*; *undefined*) for **Crown** (Figure 2).

Agglomerative hierarchical clustering

Morphological data

The different clusterings of the same morphological data, MORPH106, yielded partially different classifications of the same samples. When the data were classified using the UPGMA Method, setting the proximity measure as the Jaccard Index, and then classified using Ward's Method with the proximity measure set to Euclidean distance, and truncating both procedures when they had produced three classes to match the three classes predicted by the current taxonomy, the two methods produced corresponding classes containing 49, 55, and 2 and 39, 64 and 3 samples respectively. When these results were crosstabulated, they produced the following contingency table (Table 6). The two classifications agree in the classification of 85 of the 106 samples (80.2%). Cohen's Unweighted Kappa is 0.616 (SE 0.0721), which is 75.4% of its maximum possible value, given the observed marginal totals. This level of agreement may exceed what would be expected by chance.

Table 6

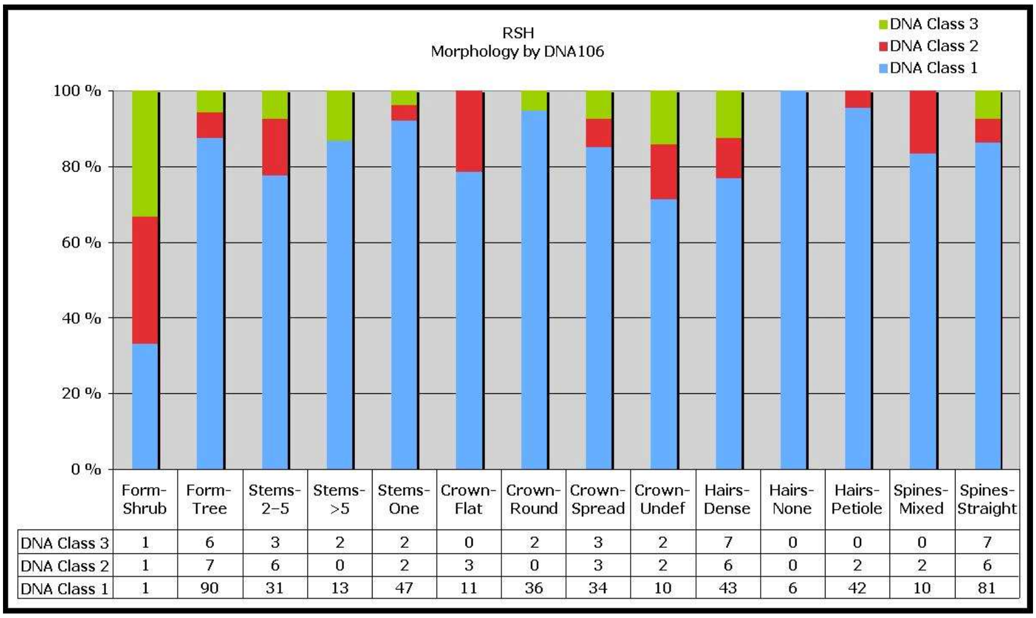
UPGMA-Jaccard and Ward Euclidean crosstabulation. Contingency table showing the relation of crosstabulation of classes produced by UPGMA-Jaccard and Ward Euclidean Methods using the morphological data of 106 samples.

MORPH106	UPGMA Jaccard Class 1	UPGMA Jaccard Class 2	UPGMA Jaccard Class 3	Total
Ward Euclidean Class 1	35	2	2	39
Ward Euclidean Class 2	14	50	0	64
Ward Euclidean Class 3	0	3	0	3
Total	49	55	2	106

Table 7

Cross-tabulation of AHC. Table showing the results of cross-tabulating the classes formed by AHC, applying various clustering techniques to either the MORPH106 or DNA106 data.

Data type	Cross-tabulation between		Number of common samples	Agreement percent	Kappa value (if possible to calculate)	Evaluation of Kappa
	Clustering method	Proximity measures				
MORPH 106	UPGMA	Jaccard index	48	45.3	Cannot be calculated	Reliability is not satisfactory
	UPGMA	Dice's Index				
MORPH 106	Ward's	Euclidean distance	34	32.1	Cannot be calculated	Reliability is not satisfactory
	UPGMA	Dice's Index				
DNA106	UPGMA	Jaccard index	106	100	1	Perfect concordance
	UPGMA	Dice's Index				
DNA106	UPGMA	Jaccard (or Dice's) index	34	32.1	0.068	Reliability is not satisfactory
	Ward's	Euclidean distance				



	Form-Shrub	Form-Tree	Stems-2–5	Stems->5	Stems-One	Crown-Flat	Crown-Round	Crown-Spread	Crown-Undef	Hairs-Dense	Hairs-None	Hairs-Petiole	Spines-Mixed	Spines-Straight
DNA Class 3	1	6	3	2	2	0	2	3	2	7	0	0	0	7
DNA Class 2	1	7	6	0	2	3	0	3	2	6	0	2	2	6
DNA Class 1	1	90	31	13	47	11	36	34	10	43	6	42	10	81

Figure 2

Chart DNA class by morphological variables. Bar chart of morphological variables cross-tabulated with the three DNA classes produced by AHC (UPGMA-Jaccard's coefficient.

Similarly, when MORPH106 was classified using the UPGMA Method, but setting the proximity measures first as the Jaccard Index and then as Dice's Index, both indices being appropriate for binary data, and again truncating for three classes, the result of their crosstabulation is that shown in Table 7. The two indices are closely related, the Dice Index being derived from the Jaccard Index, and differing only in giving a double weight to agreements (1s), which is appropriate, inasmuch as the classifications are based on characters observed to be present in the trees being classified. This time there are only 48 agreements (45.3%), and *kappa* cannot be calculated because the observed agreement is smaller than the mean-chance agreement. This degree of agreement may be due to chance.

This result is not far from what was produced when MORPH106 was classified using Ward's method with Euclidean distance and then the UPGMA Method with Dice's Index, again truncating for three classes (Table 7). This time there were only 34 agreements (32.1%), again no *kappa* is possible, and the degree of agreement may be due to chance.

DNA data

When DNA106 was subjected to the same AHC procedures as MORPH106, there were both marked divergences and marked agreements between the resultant classifications.

Notably, when DNA106 was classified by AHC using the UPGMA Method with Jaccard's and then with Dice's Index, with both set to truncate at three classes, the number of constituents of the three classes produced were exactly the same (Table 7). Cohen's *kappa* is 1.0. This agreement is greater than would be expected by chance. It is in marked contrast to what happened when the same procedures were applied to MORPH106 (see above).

On the other hand, when DNA106 was classified using the UPGMA method with either Jaccard's or with Dice's Index and the Ward method with Euclidean distance, with both set to truncate at three classes, and the resultant classes then crosstabulated, the following table was produced (Table 7). The two methods agreed in the classification of only 34 (32.1%) of the 106 samples, but appeared to disagree sharply on the status of a second and third classes in this data set. Cohen's Unweighted Kappa is

0.068 (SE 0.1196), which is 39.6% of its maximum possible value, given the observed marginal totals. This degree of agreement may be due to chance.

These results indicated that regardless of which clustering method was applied, the reliability of the method was not satisfactory; and the agreements may have been obtained by chance.

DNA and Morphological Data

When different morphological and genetic classifications of the same dataset were compared, the same discord already noted persisted.

When the AHC classification of DNA106, using Ward's method and Euclidean distance and truncating for 3 classes, was crosstabulated with the same AHC of MORPH106, the following table was produced (Table 8). There were 51 agreements (48.1%) between the two classifications, and Cohen's kappa had a value of 0.2418 (SE 0.0819), which is 52.3% of its maximum possible value, given the observed marginal totals. This level of agreement does not exceed what might arise by chance.

When the AHC classification of DNA106, using the UPGMA method and Jaccard's Index, and truncating for three classes, was crosstabulated with the same AHC of MORPH106, the following table was produced (Table 8). There were 52 agreements (49%) between the two classifications, and Cohen's kappa had a value of 0.0947 (SE 0.1342), which is 44.7% of its maximum possible value, given the observed marginal totals. This level of agreement does not exceed what might arise by chance.

When the AHC classification of DNA106, using the UPGMA method and Dice's Index, and truncating for three classes, was crosstabulated with the same AHC of MORPH106, the following table was produced (Table 8). This time there were 89 agreements (83.95%) between the two classifications, and Cohen's kappa had a value of 0.1021 (SE 0.1229), which is only 21.6% of its maximum possible value, given the observed marginal totals. This is the greatest agreement between any two classifications hitherto compared, but the value for kappa is still so low that even this degree of agreement might be due to chance. This is similar to what obtained using CHAID (Table 5).

Table 8
AHC DNA106 by MORPH106. Table showing the results of cross-tabulating the classes formed by AHC, applying various clustering techniques to the MORPH106 verses DNA106 data using the same technique.

Cross-tabulation between		**Number of common samples**	**Agreement percent**	**Kappa value (if possible to calculate)**	**Evaluation of Kappa**
Clustering method	**Proximity measures**				
Ward's	Euclidean distance	51	48.1	0.2418	Reliability is not satisfactory
UPGMA	Jaccard index	52	49	0.09	Reliability is not satisfactory
UPGMA	Dice's Index	89	83.95	0.1021	Reliability is not satisfactory

Table 9
Morphological variables by DNA classes. Chi-square test relating morphological variables to the three DNA classes.

	Spines	Stems	Crown	Hairs	Form
Chi-square (Observed value)	2.395	7.159	10.391	9.121	7.052
Chi-square (Critical value)	5.991	9.488	12.592	9.488	5.991
DF	2	4	6	4	2
p-value	0.302	0.128	0.109	0.058	0.029
alpha	0.05	0.05	0.05	0.05	0.05

When the variables in MORPH106 are crosstabulated with the three DNA classes produced by AHC (UPGMA, Jaccard's coefficient, Figure 2), there is almost no systematic relationship among them. When the contingency tables for the values of each variable and the DNA classes are tested for independence using a chi-square test, only **Growth Form** shows a slight dependence at an alpha of 0.05 (Table 9). The rows and columns of the contingency tables for the other morphological variables are statistically independent.

DNA and Morphological Comparison Visualized

An example of individuals that are morphologically different but genetically similar is illustrated in Figure 3. Photographs of four individuals from the same locality (Nubahweeb in Sudan) are displayed.

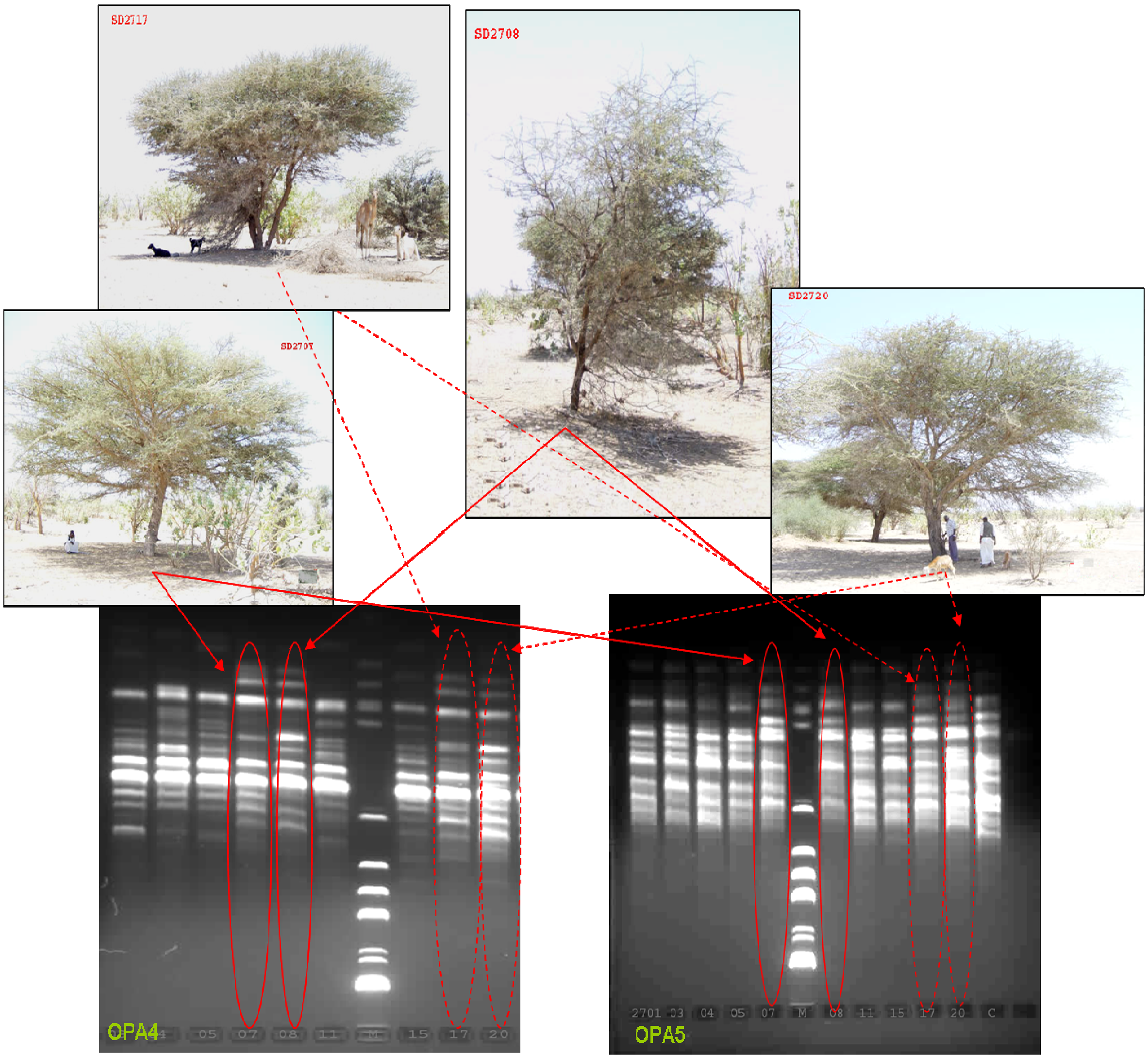


Figure 3
Habit of four plants from Nubahweeb, Sudan, comparing the DNA patterns of morphologically different plants. They fell into the same class genetically but belonged to two different classes morphologically; each marked by solid or dotted boundaries around their DNA pattern when using two primers OPA4 and OPA5.

These individuals, when clustered using Ward's method with Euclidean distance and using program automatic truncation level, two classes from MORPH106 were formed while four were formed out of DNA106. The four individuals fell into the same DNA cluster (DNA106, class 3); but two of them (SD2707 and SD2708) fell into morphological group one (MORPH106, class 1) and the other two (SD2717 and SD2720) into group two (MORPH106, class 2). Two gel photographs of primers OPA4 and OPA5 are placed below the pictures of these trees to show the similarity of their DNA patterns. Three of these four individuals have one stem, while 2717 has the stem value 2-5. The main feature that distinguishes these individuals is the density of their hairs. Those grouped with class 1 have hair only on their petioles, whereas hairs covered all the branchlets of the other two. Dendograms representing the classes formed by DNA106 and MORPH106 is shown in Figure 4.

Therefore, all attempts to find a congruence between classes that were formed from morphological data and/or DNA data failed. It made no difference whether the data was set to be truncated into three classes, as was expected if there were three subspecies in the study area, or allowed the software to choose an automatic level of truncation. This showed that caution should be exercised when classifying plants to subspecies level using only their morphology. Other studies have reached similar conclusions about other plant~~s~~ species were also attained [17-19].

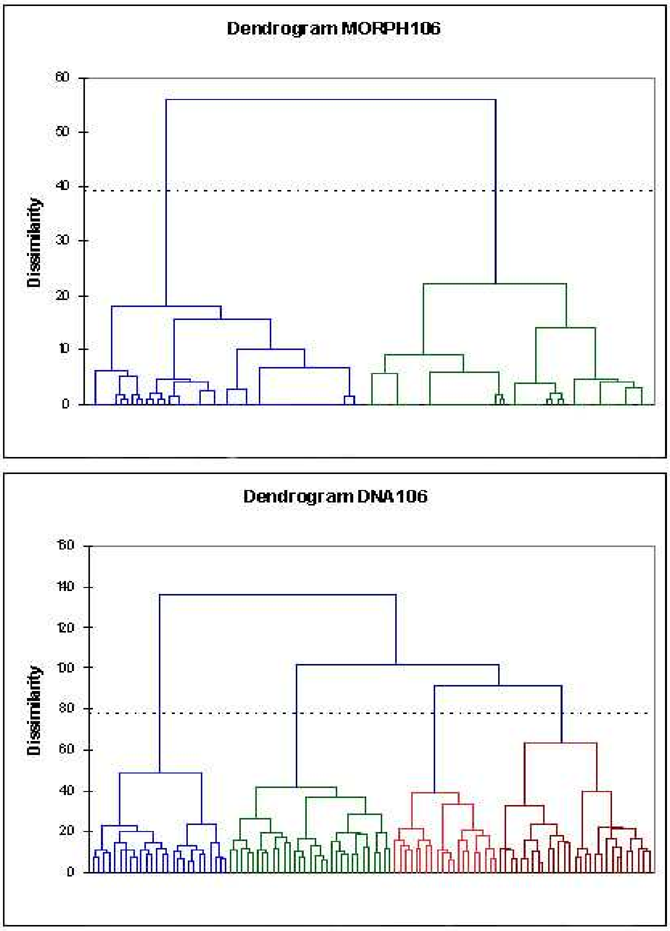


Figure 4
Dendograms of MORPH106 and DNA106. Dendograms formed when cluster MORPH106 (forming two classes) and DNA106 (forming four classes) using Euclidean distance and Ward’s method and automatic truncation.

Conclusion

The study is designed to investigate the current taxonomy of *A. tortilis* in the RSH of Sudan and Egypt. The preceding analyses tested whether the dataset of *Acacia tortilis* samples could be clustered into three classes based on "morphological" variables used in the current taxonomy that were consistent with three corresponding genomic classes based on DNA extracted from those same samples. No such correspondence emerged. The lack of a significant statistical relationship between the morphological variables and the classes of DNA suggest that the genetic structure underlying the *Acacia tortilis* group is related to as yet unspecified factors. The doubts expressed at the outset of this study about the current taxonomy have not been dispelled.

Materials and methods

Localities studied and the collection of samples

A total of 33 localities in the RSH in Sudan and Egypt were examined in the period from February to April 2003 (AbdElRahman & Krzywinski "unpublished data"). Of these 20 were subjected to closer scrutiny within the frame of reference of this paper. Names of the khors sampled and number of samples used in this study are tabulated in Table 10. For the purposes of this paper, as well, 106 samples (Sudan = 80 and Egypt= 26) were drawn from a larger set of 520 samples so as to represent the DNA from plants selected from the three *A. tortilis* sub-taxa reported in the study area. These 106 samples were chosen because both morphological data and molecular data (cf. section Data Analysis) were available for analysis. The geographic coordinates of each sample were also recorded and used to fix the positions of the localities (Figure 1).

Data description

Morphological features

The morphological characters of the samples used in the present data analysis were recorded on site and are similar to those which have been used in relevant literature to identify the sub-taxa of *A. tortilis* [10-12, 20-27]. They were coded as 1 or 0 for the presence or absence of the variable in the plant observed. These variables (**shown in bold**) represent:

- growth form (**tree /shrub**);
- number of stems from ground

level (**stem = 1/ stem = 2 – 5 / stem > 5**);

- distribution of hairs when seen by the naked eye (**no hair / only in the petiole / young branchlets densely pubescent**), i.e., the plant is glabrous / sub-glabrous / pubescent respectively as indicted in Table 1;
- the shape of the crown (**round / flat / spread / undefined**);
- spine shape (**spine = straight / spine = mixed**, i.e., both straight and curved).

Table 10
Regions, localities, and number of samples

Region	Location name	no. of samples
Egypt	Wadi Jimal 1	1
	Wadi Jimal 2	1
	Wadi Nuqrus	7
	Wadi Sukkari	3
	Wadi Sartut 2	3
	Wadi Hulayfi	3
	West Wadi Hanjaliyyah	3
	Wadi Muylhie	5
Sudan	Upper Beramio	9
	Lower Beramio	8
	Upper Amat	7
	Lower Amat	6
	Upper Hilayet	11
	Nubahweeb	8
	Upper Biramfi	6
	Dybadwat	7
	Lakatyeb	6
	Upper Sarara Agwampt	3
	Lower Sarara Agwampt	3
	Mid Sarara Agwampt	6

Methods

Data collection

Acacia leaves were collected in the field, wrapped in gauze, labelled and placed in plastic bags filled with silica gel [28]. Then they were shipped to The University of Bergen, Biology Department where the laboratory analysis for this study was performed.

DNA extraction

The extraction was done using the DNeasy® Plant Mini Kit in conformity with the procedures specified in the accompanying handbook. The work was done in batches, and at each step in the procedure care was taken to avoid contaminating the samples.

Molecular polymorphism

The DNA amplification was based on PCR-RAPD technique [29]. The procedure followed was: a dilution of 1/50 of the extracted DNA was used for the DNA amplification, which was performed using Polymerase chain reaction (PCR) on a GeneAmp® PCR System 2700 machine from Applied Biosystems. The PCR setup was as follows: all the reagents were incubated in ice throughout the performance of the reaction. To 5 µl of the diluted DNA extract, a total of 20 µl of the following mixture was added [deionised water (10.95 µl), klen buffer (2.5 µl, DNA Polymerase Technology), Magnesium chloride (1 µl), dNTPs (2 µl), primer (3.5 µl) and Klen-Taq (0.05µl)]. The optimal procedures followed were: initial cycle at 94°C for 3 min, followed by 42 cycles at 94°C for 20 s each, then a cycle at 36°C for 30 s, followed by a further cycle at 72° for 2 min; and a final cycle at 72°C for 7 min. The samples were subsequently kept at 9°C until they were removed from the PCR machine. Twenty-six 10-mer primers (nucleotide sequence 5´to 3´: GTA GAC CCG T; TGC CGA GCT G; AGT CAG CCA C; AAT CGG GCT G; AGG GGT CTT G; GGT GCG GGA A; GTT TCG CTC C; AAG AGC CCG T; ACC GCE CAA C; CCG GTC AGC A; CAG GCC CTT C; GGT CCC TGA C; GAA ACG GGT G; GTG ACG TAG G; GGG TAA CGC C; GTG ATC GCA G; CAA TCG CCG T; TCG GCG ATA G; CAG CAC CCA C; TCT GTG CTG G; TTC CGA ACC C; AGC CAG CGA A; GAC CGC TTG T; AGG TGA CCG T; CAA ACG TCG G; GTT GCG ATC C) were evaluated but only first five successfully produced polymorphic and reproducible bands among the samples studied.

Loading of the samples for gel electrophoresis

Before loading the samples onto the gel, they were centrifuged at 8000 x g. To 3µl of the loading buffer, 12µl of the PCR product was added. DNA amplification fragments were separated in 2% agarose gel in a 1XTBE (Tris–boric acid-EDTA) buffer. The gels were stained with ethidium bromide (1:10000 $EtBr_2$: Agarose gel) and visualized on an UV transilluminator. Depending on the size of the agrose gel, the reaction was run at 80 V for at least three hours. Then, using the image acquisition software GeneSnap, the gel was visualized and photographed. Each amplification reaction was performed using a single primer. Twenty six 10-mer primers were evaluated, of which five yielded reproducible bands across all 106 samples. One hundred and ten bands of PCR fragments were revealed from all samples and were scored as 1 for the presence of a given band and 0 for its absence.

Data analysis

Construction of matrices

Two binary matrices were constructed, one denoting the presence or absence in each sample of the morphological characters observed and recorded in the field and the other denoting the presence or absence of distinctive DNA bands extracted from those samples in the DNA Laboratory of the Biology Department at the University of Bergen. In addition, a nominal contingency table of the morphological data was constructed (hereafter referred to as MORPHNOM520). Subsets of this table are distinguished by a numerical suffix denoting how many samples each contains, e.g. MORPHNOM106.

Because the morphological data are categorical, polythetic and rely on the observation of the presence or absence of specific characters (variables) rather than on instrumentation and because several of these characters admit of more than two values, this data was coded for a binary *disjunctive* table (MORPH106). Subsets of this table are distinguished by a numerical suffix denoting how many samples each contains, e.g. MORPH105.

Because the DNA data consist in observations of the presence or absence of 110 specific bands of DNA (their numbering runs from 1 to 120, but ten bands are irrelevant for the present analyses and have been omitted) none of which have more than two values, this data is constructed as a binary *contingency* table (hereafter DNA106). Subsets of this table are distinguished by a suffix denoting how many samples each contains, e.g. DNA105.

Chi-square Automatic Interaction Detection (CHAID)

To investigate the effect of the different variables on the classifications of the samples, MORPH520 was run in CHAID [30] using the samples as the qualitative dependent variable and the characters as the qualitative explanatory variables. The measure used was the Pearson Chi-square, the maximum tree depth was set at 5, the significance level and the split threshold were set at 0.05, the minimum parent size allowed was 2, and the minimum son size allowed was one. The implementation of CHAID used for this study was that provided in XLSTAT.

Agglomerative hierarchical clustering (AHC)

Agglomerative Hierarchical Clustering (AHC) was employed to classify the samples recorded in the tables. Since different clustering methods applied to the same dataset can yield different results, two clustering methods were tested, the Unweighted Pair Group Method with Arithmetic mean (UPGMA) and Ward's Method. Since different measures of proximity yield different results when used with the same clustering methods and since the data used in this procedure are represented by binary coding, different measures were tested to compute the proximity of the samples. These were primarily the Jaccard Index and the Dice Coefficient, though Euclidean distance was regularly used for Ward's Method. The two former are related, the latter of the two giving double weight to agreements between observations. As a rule these procedures were truncated for three classes, but were sometimes also set to generate four, five, and greater than five classes when testing for different outcomes. For visualization of the difference between classes formed by DNA106 and MORPH106, each of the two datasets was analysed as follows: using Euclidean distance as the dissimilarity coefficient and Ward's method as the agglomeration method [31]. Ward's method minimizes the within class variance. This time the truncation was set to be automatic. Here too the implementation used was that provided in XLSTAT.

Crosstabulation and Comparison

To compare the classifications

obtained from different clusterings of MORPH and DNA, they were crosstabulated into two-way contingency tables using XLSTAT and then tested using Cohen's *Kappa*. This measure ranges between 0 and +1.0. The nearer *kappa* approaches +1.0 the less likely it is that, given the observed marginal totals, the agreement between *two* classifications is due to chance. *Unweighted kappa* is the appropriate form of *kappa* for nominal categories, which are what is discussed in this paper. Although *kappa* is widely used, it is controversial not least when applied to skewed data, as is some of the data presented here, and particularly as regards its use to quantify levels of agreement. Here it is used to assess whether agreement exceeds chance levels. There is no assured value of *kappa* below which it is agreed to accept the null hypothesis that an observed agreement is due to chance, but conventionally a value of kappa above 0.7 may indicate that a degree of agreement is not due to chance. *Kappa* was computed using the implementation made available at http://faculty.vassar.edu/lowry/kappa.html.

List of Abbreviations

ACH = Agglomerative Hierarchical Clustering

CHAID = Chi-square Automatic Interaction Detection

PCR = Polymerase chain reaction

RAPD = Random Amplified Polymorphic DNA

RSH = Red Sea Hills

UPGMA = Unweighted Pair Group Method with Arithmetic Mean

Author's contribution

All authors conducted the field, designed the analysis and approved the final manuscript. HFA and RHP performed the statistical analysis.

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Paper III

Howaida Faisal AbdElRahman, Knut Krzywinski and Stefan Ekman. **Genetic Diversity and Population Differentiation in the *Acacia tortilis* Group of the Red Sea Hills, Southeast Egypt and Northeast Sudan**. (Manuscript to be submitted).

Genetic Diversity and Population Differentiation in the *Acacia tortilis* Group of the Red Sea Hills, Southeast Egypt and Northeast Sudan

Howaida Faisal AbdElRahman*[1], Knut Krzywinski[1] and Stefan Ekman[2]
[1]Department of Biology, University of Bergen, P O Box 5007, Bergen, Norway and [2] Museum of Evolution, Evolutionary Biology Centre, Uppsala University, Norbyvägen 16, SE-75236 Uppsala, Sweden
Email: Howaida F AbdElRahman* - howaida.abdelrahman@bio.uib.no; Knut Krzywinski knut.krzywinski@uib.no; Stefan Ekman - stefan.ekman@evolmuseum.uu.se
* Corresponding author.

Abstract

Levels of gene flow, population structure and the correlation between structure and current taxonomy in *A. tortilis* along a distinct environmental gradient along the Red Sea Hills of Sudan and Egypt were studied using Random Amplified Polymorphic DNA (RAPD). Analysis of Molecular Variance (AMOVA), grouping individuals by geographic origin, indicated that regions (Sudan, Egypt) as well as populations within regions were significantly different. An alternative AMOVA, grouping individuals by their subspecific identity, indicated that a very small amount of variance is between groups (subspecies). An exact test of population differentiation showed no significant difference between putative subspecies of *A. tortilis*. A Mantel test indicated significant isolation-by-distance between populations. A cluster analysis displayed consistent genetic differences between the Egyptian and Sudanese populations sampled. It is concluded that the three subspecies into which *A. tortilis* is traditionally classified should probably not be recognized as taxa and that gene flow between populations seems to be restricted.

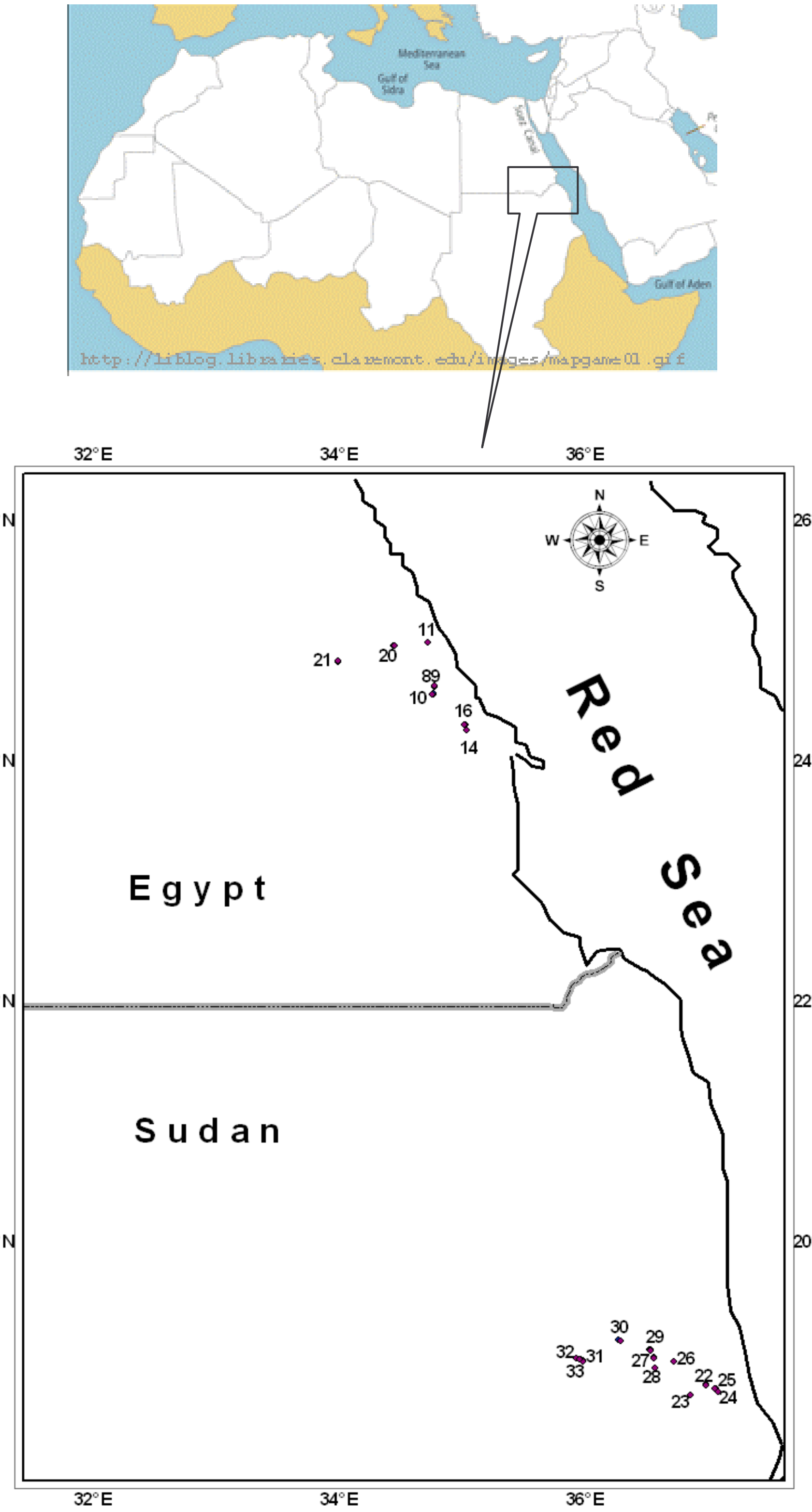
Introduction

In arid regions, trees play an important role as the only source of food for livestock and shade and firewood for humans. *Acacia tortilis* is one of the most common arboreal species surviving the harsh environmental conditions of the arid environment in North Africa [1-6]. The frequent years of drought, low rainfall and extreme hot temperatures make the arid land of North Africa vulnerable to climate change (UNESCO [7]). The wadi ecosystems that host arboreal vegetation represent a contracted pattern as a reminiscence of once continuous savannah vegetation during the Mid-Holocene. As a result of desiccation since this moister period, plant species were restricted to small isolated populations in favourable locations, particularly in the dry river valleys. Here they take advantage of subsurface moisture as well as upstream dewfall and scattered rainfall. The number of *A. tortilis* individuals has in places been severely reduced owing to environmental changes as well as anthropogenic activities [6, 8, 9]. In the study area, the Red Sea Hills of Egypt and Sudan, grow what have been considered as three subspecies, *A. tortilis* ssp *raddiana*, ssp *tortilis*, and ssp *spirocarpa* [10-12]. Genetic variation in the *A. tortilis* has been shown to be uncorrelated with current subspecies taxonomy of the complex as described in existing floras (AbdElRahman et al. submitted manuscript). A previous study of genetic variation in *A. tortilis* from the Negev Desert concluded that general population differentiation of *A. tortilis* (referred to as *A. raddiana*) was considerably greater than that recorded for other outcrossing plant taxa as determined by random polymorphic DNA analysis, and that the western Negev and Arava Valley populations are highly differentiated [13]. The objectives of the current study were to infer levels of gene flow, population structure and the correlation between structure and current taxonomy in *A. tortilis* along a distinct environmental gradient along the Red Sea Hills of Sudan and Egypt. We used the Random Amplified Polymorphic DNA (RAPD) technique which is useful for identifying polymorphism and for studying the population genetic structure of organisms [14]. Moreover, it is technically straightforward, applicable to any organism without previous knowledge of its genome, and allows the screening of a high number of polymorphic markers for genetic population studies [15].

Materials and methods

Sampling

Leaves from the three reported subspecies of *A. tortilis* were collected from 19 populations in the Red Sea Region of Sudan (12 populations) and Egypt (7 populations) (Figure 1) (Table 1). A population is a group of individual organisms of the same species in a given area [16]. Here the term population means collective individuals growing in the same locality. Pairwise geographical distances between populations ranged from 2.5 km (Upper Sarara Agwampt & Mid Sarara Agwampt) to 680 km (Mumaylhah & Upper Amat). Distances were calculated using ArcGis version 9.1. The total number of plants from which leaves were collected was 158, of which 28 were from Egypt and 130 from Sudan. The number of individuals per population ranged from 3 to 25. If possible, leaves from the youngest branchlets were collected. They were wrapped in gauze, labelled and kept in plastic bags with silica gel [17]. Laboratory analyses were carried out at the Department of Biology, University of Bergen.



http://liblog.libraries.claremont.edu/images/mapgame01.gif

Fig. 1. Study area. A map showing the location of the study area in relation to North Africa (up). Geographical positions of the populations studied are shown by the small-red circles (down).

The following morphological characters were recorded in field for each sample collected: growth form (tree or a shrub), the number of the stems above ground (1, 2-5, or more than 5); the distribution of the hair as seen by the naked eye (no hair, hairs only on the petiole, or all over the young branchlet); and crown shape (round, flat, flat-spreading or irregular). These morphological characters are those used in the existing floras to distinguish the various sub-taxa of *A. tortilis* [10-12, 18]. Accordingly, trees having one stem, rounded crown and lacking hairs were identified as spp. *raddiana*; small trees or shrubs with more than five stems, flat crown and hairs covering the young branchlets as spp. *tortilis*, and trees having 2-5 stems, flat-spreading crown, and hairs either on the petiole or over the young branchlet as spp. *spirocarpa*. When these characters overlap the plant was treated as unclassified.

Table 1. Code, name, location, elevation and number of samples collected from each population.

Population code (as it appears in the map of Fig.1)	Population name	Geographic position		Elevation /m.a.s.l	Number of samples analysed
		E	N		
89	Jimal	34° 45'	24° 33'	285	3
10	Nuqrus	34° 46'	24° 37'	304	7
11	Sukkari	34° 43'	24° 59'	300	3
14	Sartut	35° 01'	24° 17'	347	4
16	Hulayfi	35° 02'	24° 15'	513	3
20	Hanjaliyyah	34° 26'	24° 57'	565	3
21	Mumaylhah	33° 59'	24° 49'	358	5
22	Upper Beramio	36° 58'	18° 47'	1052	14
23	Lower Beramio	36° 51'	18° 42'	920	25
24	Upper Amat	37° 05'	18° 44'	1079	12
25	Lower Amat	37° 03'	18°46'	1018	14
26	Upper Hilayet	36° 43'	18° 59'	973	13
27	Nubahweeb	36° 33'	19° 01'	883	10
28	Upper Beramfi	36° 33'	18° 56'	926	7
29	Dybadwat	36° 31'	19° 05'	816	7
30	Lakatyeb	36° 17'	19° 10'	816	8
31	Upper Sarara Agwampt	35° 58'	18° 59'	642	5

32	Lower Sarara Agwampt	35° 55'	19° 01'	611	5
33	Mid Sarara Agwampt	35° 57'	19° 00'	631	10

DNA extraction and PCR amplification

Approximately 2 mg of dried leaves proved to be the optimal amount for DNA extraction. This amount was weighed in a sterile plastic microcentrifuge tube and then the tube was placed in liquid nitrogen to freeze the leaves. A sterile plastic pestle was used to grind the frozen leaves. Total DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN) following the manufacturer's protocol. DNA was eluted with H_2O to a final volume of 200 µl and 23 µlbuffer TE (pH 8.3) added.

A dilution of 1/50 of the extracted DNA was used for the polymerase chain reaction (PCR). PCR was performed in a total volume of 25 µl. All reagents were kept on ice throughout the preparations of the PCR. To 5 µl of the diluted DNA extract, a total of 20 µl of the following mixture was added: deionised water (10,95 µl), klen buffer (2,5 µl), magnesium chloride (1 µl), dNTPs (2 µl of each), primer (3,5 µl), the polymerase Klentaq (0,05µl; DNA Polymerase Technology). DNA amplification was in the same GeneAmp 2700 PCR machine (Applied Biosystems). The following PCR cycling conditions were adhered to: An initial hold at 94°C for 3 minutes, followed by 42 cycles of 94°C for 20 seconds, 36°C for 30 seconds, and 72° for 2 minutes; and finally a hold at 72°C for 7 minutes before the reaction was cooled to a constant 9°C.

Twenty-six 10-mer primers (Table 2) were evaluated but only five successfully produced polymorphic and reproducible bands among the samples studied.

Table 2. Nucleotide sequence 5´to 3´ of the tested primers. The nucleotide sequence of the 26 10-mer primers tested, only the five first on the list giving reproducible polymorphic bands.

primer	nucleotide sequence 5´to 3´
RAPD 3	GTA GAC CCG T
OPA-2	TGC CGA GCT G
OPA-3	AGT CAG CCA C
OPA-4	AAT CGG GCT G
OPA-5	AGG GGT CTT G
RAPD 1	GGT GCG GGA A
RAPD 2	GTT TCG CTC C
RAPD 4	AAG AGC CCG T
RAPD 5	ACC GCE CAA C

RAPD 6	CCG GTC AGC A
OPA-1	CAG GCC CTT C
OPA-6	GGT CCC TGA C
OPA-7	GAA ACG GGT G
OPA-8	GTG ACG TAG G
OPA-9	GGG TAA CGC C
OPA-10	GTG ATC GCA G
OPA-11	CAA TCG CCG T
OPA-12	TCG GCG ATA G
OPA-13	CAG CAC CCA C
OPA-14	TCT GTG CTG G
OPA-15	TTC CGA ACC C
OPA-16	AGC CAG CGA A
OPA-17	GAC CGC TTG T
OPA-18	AGG TGA CCG T
OPA-19	CAA ACG TCG G
OPA-20	GTT GCG ATC C

Gel electrophoresis and scoring of bands

Amplified fragments were separated in 2% agarose gel in 1X TBE (Tris–boric acid-EDTA) buffer and visualized using ethidium bromide in an UV transilluminator. Bands were scored as 1 for present and 0 for absent. Bands were matched by comparing calculated molecular weights of the bands using the software GeneTool version 3.06 and by comparing photos printed in large scale. A binary matrix was prepared from the presence-absence data of bands.

Data analysis

Population genetics calculations were performed using Arlequin version 3.1 [19]. Genetic structure among populations was investigated using analysis of molecular variance (AMOVA). Two different AMOVAs were performed. In the first analysis, individuals were grouped as populations according to their geography; i.e., individual plants growing on one locality were grouped into a population (Table 1) and these populations were further classified into two groups according to the region, i.e. Sudan or Egypt. In the second analysis, individuals were grouped according to a morphological classification into subspecies. The latter analysis was performed to see if described subspecies are supported by the genetic variation patterns of the individuals studied. Individuals were classified as follows: spp. *raddiana*, spp. *tortilis*, spp. *spirocarpa* or unclassified when the morphological characters used to classify the sample overlap. In both runs, the number of permutations for significance testing was set to 10000. An exact test of population differentiation [20] was performed to test if there was any statistically significant differences among between the four classes of *A. tortilis*. This test was set as follows: length of Markov chains was 100000; number of dememorizations steps were 10000; and the significance level was 0.05. The null hypothesis was no differentiation among the four classes.

Mantel tests [21] were used to check for an association between the genetic distance (Slatkin's linearized F_{ST}) between populations and the natural logarithm of their physical distance [22]. Three tests were performed, one with all populations and two with each regions separately (Egypt, Sudan).

An attempt was made to cluster individuals by genetic data. Samples from Egypt and Sudan were treated as one dataset. Within this dataset, an attempt was made to cluster individuals 2-8 classes using R software version 2.5.0 [23] and to map these classes according to the geographical coordinates of the samples that comprised each class. In R, the software default (average with the Euclidean distance) was used to perform the Agglomerative Nesting Hierarchical Clustering.

A cluster analysis of the populations was performed using their pairwise genetic distances, which is measured as the squared Euclidean distance created by Arlequin as an output from the first AMOVA test, with an unweighted pair group method with arithmetic mean (UPGMA) in PAUP 4.0b10 [24].

Results

Population pairwise differences

The five primers revealed 114 bands, ranging from 19 to 26 bands per primer. The number of polymorphic sites displayed per population ranged from 27 (Sukkari) to 90 (Lower Beramio and Low Amat), with a mean of 55.6 (Table 3). The average number of pairwise differences within population ranged from 35.3 (Low Amat) to 18.0 (Sukkari), whereas the average number of pairwise differences between populations ranged from 42.6 (Upper Hilayet and Wadi Jimal) to 20.1 (Mumaylhah and Hanjaliyyah).

Table 3. Number of polymorphic sites per population.

Population number	Population name	No. of polymorphic sites
1	Wadi Jimal	31
2	Wadi Nuqrus	56
3	Wadi Sukkari	27
4	Wadi Sartut	40
5	Wadi Hulayfi	37
6	Wadi Hanjaliyyah	29
7	Wadi Mumaylhah	38
8	Upper Beramio	72
9	Lower Beramio	90
10	Upper Amat	82
11	Lower Amat	90
12	Upper Hilayet	73
13	Nubahweeb	82
14	Upper Beramfi	72
15	Dybadwat	56
16	Lakatyeb	42
17	Upper Sarara Agwampt	39
18	Lower Sarara Agwampt	66
19	Mid Sarara Agwampt	35

AMOVA

Comparison of the genetic variation among the population of A. tortilis

Fixation indices (Table 4) reveal large amounts of within-population variation ($F_{ST} = 0.31739$). Slight differences were observed among individuals growing on the two regions studied ($F_{CT} = 0.13741$), and among populations within a region ($F_{SC} = 0.20865$). Regions as well as populations within regions were significantly differentiated ($p = 0.00000 \pm 0.00000$).

Table 4. AMOVA test showing the results of the examining the variation among the populations of *A. tortilis*. Samples studied were grouped as populations according to the locality from which the samples were collected and further these populations were grouped into two groups according to the region, i.e. Sudan or Egypt

Source of variation	Percentage of variation
Among regions	13.74
Among populations within regions	18.00
Within populations	68.26

Comparison of the genetic variation among the infraspecific taxa of A. tortilis

When the samples of *A. tortilis* studied were grouped according to their identified subspecies of *A. tortilis*, the AMOVA test showed that the percent of variation was low (4.02%) among the "subspecies" (table 5). Fixation index ($F_{ST}= 0.04015$) revealed very small differences within the "subspecies" analysed. "Subspecies" were significantly different ($p = 0.00000 \pm 0.00000$).

Table 5. AMOVA test of the infraspecific taxa of *A. tortilis*

Source of variation	Percentage of variation
Among subspecies	4.02
Within subspecies	95.98

The exact test of sample differentiation based on haplotype frequencies accepted the null hypotheses of no subspecies differentiation (Markov chain length: 100000 steps; number of dememorizations steps: 10 000); p-value= 0.05) [25] (table 6) .

Mantel

The Mantel test was significant at $p = 0.050$; the correlation coefficients when all the populations were analyzed as one set of data and when the populations from each region were analyzed separately are shown in table 7. The correlation coefficients of the populations of the whole dataset and of the populations studied from Sudan indicated the existence of a positive significant correlation ($p = 0.0000$) between genetic and physical distance between populations, 0.51 and 0.41, respectively. In the Egyptian populations, the correlation was positive but non-significant. Geographical distances explained 26 % and 17% of the variation in genetic distance between all populations and Sudanese populations respectively.

Table 6. Differentiation test between all pairs of samples showing the non-differentiation exact P values (significance level=0.0500).

	spp. *tortilis*	spp. *raddiana*	spp. *spirocarpa*
spp. *raddiana*	1.00000 ± 0.0000		
spp. *spirocarpa*	0.33722±0.0119	0.25400±0.0209	
unclassified	1.00000±0.0000	1.00000±0.0000	0.22756±0.0215

Table 7. Outcome of the Mantel tests when the populations from both regions were analyzed as a single data set as well as each region separately.

Populations	Correlation coefficient	Determination of the genetic difference by ln physical distance (%)	Significance test	F_{ST}
All populations	0.51	0.26	0.0000	0.31739
Sudan	0.41	0.17	0.0000	0.21509
Egypt	0.02	0.0004	0.44	0.2010

Cluster analysis

The UPGMA cluster analysis revealed that the Sudanese populations and the Egyptian populations were distinctly separated into two clusters (Fig. 2).

When clustering the samples studied into several classes (ranging from 2 to 8) using R, the Sudanese samples could be clustered into several classes (from 2 to 8 classes were tried), while the Egyptian samples formed a single class in all trails. To illustrate this, an example of such classes is shown in Fig. 3 where the Sudanese samples were aggregated into three classes when R was set to provide a maximum of 3 classes. The three classes were chosen to be compared to the three reported subspecies in the study area.

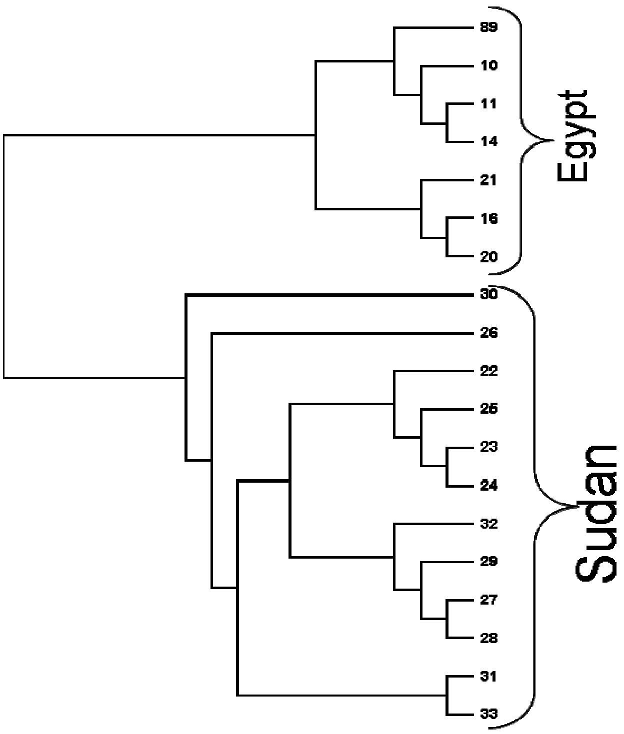


Figure 2. Population clustering using UPGMA. Cluster analysis of 19 isolated populations of *Acacia tortilis*. Clustering was performed in PAUP using the pairwise genetic distance between populations calculated by Arlequin. Number at each node correspond to population name in table 1

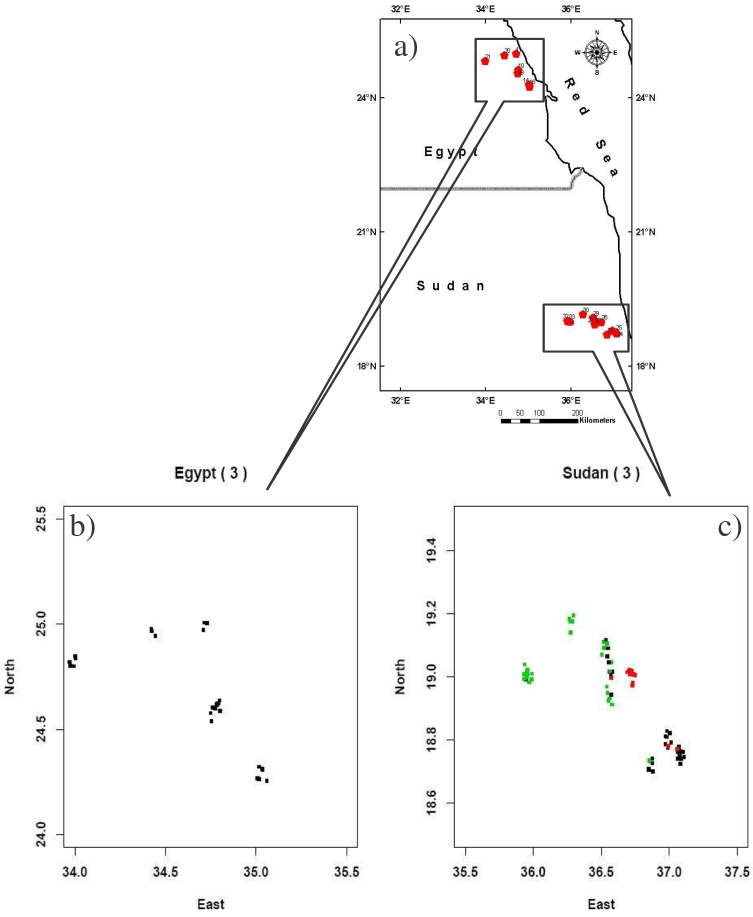


Fig. 3. Grouping of *A. tortilis* individuals, based on their genetic data using Agglomerative Nesting Hierarchical Clustering embedded in R-software, into three classes (indicated by the various colours). a) whole study area (cf. Fig.1, red polygons represent localities studied), b & c) map of Egypt and Sudan respectively including individuals studied in each region and the colours indicate their class belonging.

Discussion

The current study is an attempt to examine the level of gene flow, population structure and the correlation between structure and current taxonomy of *A. tortilis* populations along different environmental gradient of Red Sea Hills of Egypt and Sudan. The geological history of North Africa shows changes in the climate that took place over thousands of years [26, 27]. During the late Pleistocene and Holocene, there were climate changes that included an interpluvial wet period between 12000 – 6000 BP when savannah conditions prevailed across Sahara. Consequences of this climate changes was that vegetation had to shift northwards [28]. The *Acacia* species that are found in Israel are believed to be of African origin, having migrated northwards during the Holocene period [13]. The wet period was followed by an abrupt drying at 5500 BP, and deserts replaced the open savannah forest. These changes had considerable effects on the vegetation and its dynamics; many species became extinct, migrated or became isolated in patches instead of forming a continuous forest as they once had. Among the species that managed to survive these changes was *A. tortilis*. Instead of forming a continuous forest, it formed a contracted pattern of isolated populations. Individual trees of *A. tortilis* become from 200 up to 650 years and regeneration is very low [6]. The *A. tortilis* populations of the Red Sea Hills are endangered due to human activities. Gene flow between the isolated populations seems to be restricted (first AMOVA analysis, Mantel)) and has probably been so for nearly up to six thousands years. These isolated populations may be considered as remnant, long-lived populations with low recruitment.

The three infraspecific taxa of *A. tortilis*, which have been claimed to be separated by morphological characters [10-12, 18, 29] showed high genetic polymorphism which could not explain the current taxonomy (AbdElRahman HF , et. al, submitted manuscript). The very low among-subspecies variance (4%) (second AMOVA analysis) was statistically significant but the exact test of sample differentiation proved no “subspecies” differentiations. However, if the putative subspecies represented distinct taxa, one would expect a much higher among-subspecies variance. Therefore, the separation into three subspecies, as currently understood, seem not to be supported by available genetic data.

The shifting balance between the two opposing forces of genetic drift and gene flow plays a great role in the degree of genetic diversity within and between populations. While genetic drift tends to reduce diversity within the populations but increases it between them depending on the balance between drift and mutation, gene flow tends to increase the genetic diversity within a population by bringing new genes through migration of species and thus reduces the diversity between populations. The genetic difference between the two regions can be explained by two reasons, one is the isolation-by-distance due the large distance between the Egyptian and Sudanese regions (ca. 680 km) (UPGMA clustering in PAUP, Fig.2) [22]. Isolation-by-distance implies that gene flow is restricted and mainly occurs at relatively short distances (Mantel test). A second reason why Egyptian and Sudanese samples appear to be different in our analysis is that there may be unrecognized taxonomic entities which are more or less reproductively isolated. The study area is governed by a wadi drainage system (Fig. 4). It is along this drainage system that the *A. tortilis* grows. The high mountain and plateaus isolate the populations of *A. tortilis* in each wadi and limit the dispersal of seeds and pollination between neighbour populations. The Mantel test shows that the genetic difference in the Sudanese populations is correlated to the physical distance between them (Table 7). The genetic difference in the Egyptian populations shows less correlation to their physical distance and individuals form only one class clustered using R-software. This may be attributed to the fact that the number of the samples analysed from Egypt was smaller than from Sudan.

Seed dispersal is partly by wind, by water (only downstream and during flood events and by animals (goats and camels that may roam between wadis). Desert dwellers and their animals follow the wadis, whereas mountainous areas hinder them in travelling to places even at short geographic distance. Factors related to tribal organisation and land tenure govern the pattern of migration and movement of domestic animals. Variance in the genetic /geographical distance can be linked to such migration patterns. Tribal rights and land tenure follow ancient rules. Gene flow can occur not only by the dispersal of seeds but by dispersal of pollen. However, pollination is likely to be severely limited by distance. Local climatic factors seem to regulate the timing of flowering in *A. tortilis*. Consequently, even nearby *A. tortilis* stands tend to flower at different times.

The genetic divergence of *A. tortilis* populations of the Red Sea Hills in Egypt and Sudan has implications for management and conservation of the species. Re-plantation of *A. tortilis* seeds collected randomly from one locality and to be replanted in another locality should be avoided, if local genetic uniqueness is to be preserved.

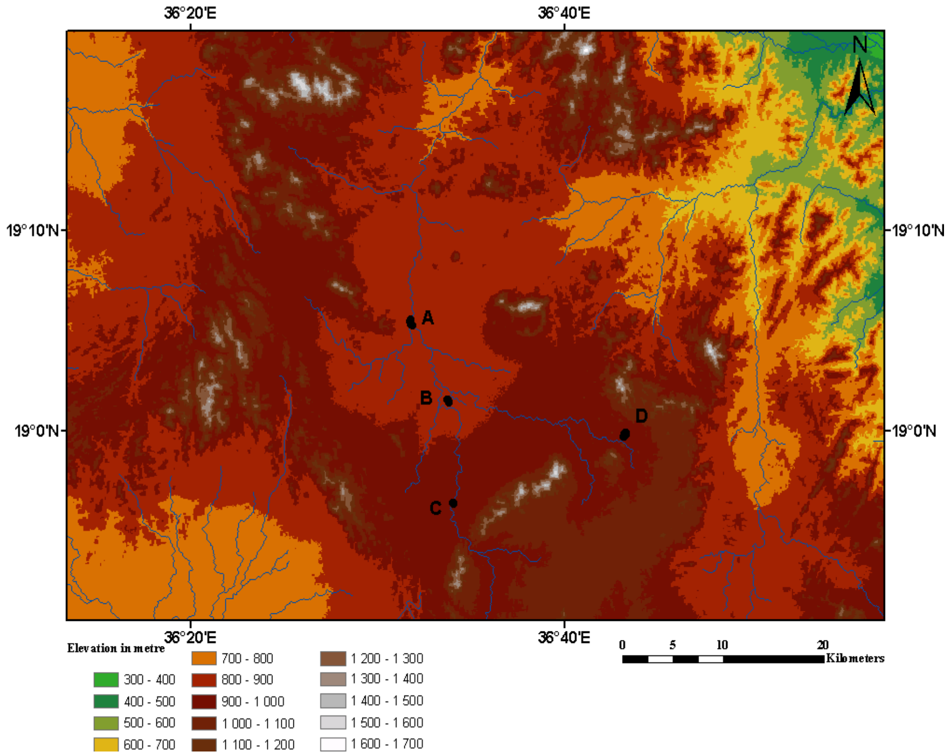


Fig. 4. The drainage system that govern the study area. The map, based in digital elevation model, showing part of the Odrous plain in Sudan. Blue lines are drainage systems. Letters corresponds to four localities included in this study; A ≡ Dybadwat, B≡ Nubahweeb, C≡Upper Beramfi and D≡ Upper Hilayet

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Paper IV

Knut Krzywinski, Gidske L. Andersen, Howaida AbdElRahman. **Morphological diversity in Acacia tortilis – the effect of environmental factors, human use and management.** (Manuscript to be submitted).

Morphological diversity in *Acacia tortilis* – the effect of environmental factors, human use and management

Knut Krzywinski[1], Gidske L. Andersen[1], Howaida AbdElRahman[1]
Department of Biology, University of Bergen, P.O.BOX 7800, N-5020 Bergen

Abstract

What is currently considered a species complex of *Acacia tortilis* consists of four taxa. While *Acacia tortilis* ssp. *heteracantha* has a distinct distribution, *Acacia tortilis* ssp. *tortilis*, *Acacia tortilis* ssp. *raddiana* and *Acacia tortilis* ssp. s*pirocarpa* have overlapping distributions as well as overlapping morphological characteristics. Molecular genetic studies do not support these currently recognized infraspecific taxa. In this study, performed in south-eastern Egypt and north-eastern Sudan, it is argued that morphological variability within the North African *A. tortilis* could be attributed to a combination of natural and anthropogenic environmental pressures. Because *A. tortilis* is a key species and constitutes a major resource in arid lands, it is intensively utilised by local inhabitants. The effect of utilisation on *A. tortilis* growth forms has, however, been poorly acknowledged in existing literature. The range of growth forms and intermediate forms may result from a combination of local climate, browsing and pollarding pressures. The bush-shaped "*Acacia tortilis* ssp. *tortilis"* and multi-stemmed trees with flat crowns referred to as "*Acacia tortilis* ssp. *spirocarpa",* can be formed as a result of browsing and drought in sapling and bush stages. When trees develop into larger individuals, collecting material from them for various purpose reduces the number of stems, and combined pollarding and browsing shape their crowns. When intensity of management increases, as for instance in hyper-arid areas where other resources are nearly absent, there is a selection towards single-stemmed trees with rounded/irregular crowns, i.e. toward the growth form formerly corresponding to "*Acacia tortilis* ssp. *raddiana*".

Introduction

Acacia tortilis (Forssk.) Hayne, an ecological key species in arid and semi-arid environments in Africa and on the Sinai and Arabian peninsulas [1-4], is characterised by its long thorns and flat-topped or umbrella shaped growth form. A considerable morphological variability exists among the four currently recognized taxa (*A. tortilis* ssp. *tortilis*, *A. tortilis* ssp. *spirocarpa* (Hochst. ex A. Rich) Brenan, *A. tortilis* ssp. *raddiana* (Savi) Brenan and *A. tortilis* ssp. *heteracantha* (Burch.) [5,6,7]. Intermediate forms have been described as hybrids or variants [5-6,8]. It has long been an open question whether these taxa, collectively referred to as the *A. tortilis* complex, are separate species or subspecies [5,8,9,10].

Identification of *A. tortilis* ssp. *heteracantha* is based mainly on its distinct geographical distribution (South Africa), while differentiation among the other three taxa is less straightforward because of a considerable overlap in morphological characters as well as distribution area [5,7,11]. *A. tortilis* ssp. *spirocarpa* occur mainly in south and south-eastern Africa, with an extension into Sudan. *A. tortilis* ssp. *tortilis* and *A. tortilis* ssp. *raddiana* are widespread in northern Africa and on the Sinai and Arabian peninsulas, with a significant overlapping distribution on both sides of the Red Sea and on the Horn of Africa [5,7,12].

Of the characters used to distinguish among taxa are hairiness on pods and vegetative parts. It is a diffuse character that varies and overlaps considerably [5,7,13,14]. Another set of characters frequently referred to are height, number of stems and shape of crown [5,7,13,15]. Regional floras are, however, inconsistent in their descriptions of these growth form characters [16,17]. Various authors have for this reason pointed out that it is difficult to distinguish among the taxa due to lack of distinctiveness of identification characters [18,19].

Recent molecular taxonomic studies of the *A. tortilis* complex based on samples from the three taxa growing in the Red Sea Hills show considerable genetic diversity among individuals (AbdElRahman, H.F., Krzywinski K, Ekman, S., manuscript). There is, however, no convergence between the classification based on molecular data and the morphological characters used to discriminate between *A. tortilis* ssp. *tortilis*, *A. tortilis* ssp. *raddiana* and *A. tortilis* ssp. *spirocarpa* (AbdElRahman, H.F., Krzywinski K, Pierce, R.H., manuscript). While no basis for intra-specific differentiation among studied samples was found, the genetic distance among samples could be correlated to the geographical distance among populations sampled (AbdElRahman, H.F., Krzywinski K, Ekman, S., manuscript). The genetic variance seems linked to a significant isolation by distance among local populations and to restricted gene flow. As these molecular taxonomic studies indicate that the *A. tortilis* "complex" consists of only one species, the evident morphological variance within this species remains unexplained; it seems likely, however, that its morphological variability and growth forms are related to environmental factors.

Another species that develops a range of growth forms and architectures depending on habitat-type and disturbance regime is *Acacia karroo* [20]. Some of the most significant environmental factors/disturbances shaping tree growth form in arid lands are fire, droughts and browsing [20-22]. One strategy of trees to cope with such disturbance pressure is to develop high plasticity and a capability to resprout, both of which increase persistence and can result in multi-stemmed individuals [18,23]. An adaptive strategy in fire regimes seems to be fast growth, investing all energy into one shoot in order to escape the suppressed juvenile (gulliver) state and becoming a tall single-stemmed tree with a crown above the reach of bushfires and thereby avoiding topkill [20,24]. Because fires, like drought and browsing, are to a large degree stochastic disturbances, high plasticity and the ability to develop different growth forms or ecotypes within species, is expected, as e.g. for *A. karroo* [20]. Within the arid core of *A. tortilis'* distribution-area, disturbance from bush fires is insignificant; but disturbances from both droughts and browsing are frequent and do influence tree growth form, for instance by reducing biomass and inducing resprouting [18].

Because *A. tortilis* has a drought enduring survival strategy, it is a multipurpose tree that secures resource availability, in particular of fodder and fuel, for people and animals - also during prolonged rainless periods when other resources are scarce [2]. The effect of traditional tribal use and management of trees on growth forms of *A. tortilis* has, however, received little attention. In an arid land, anthropogenic factors add to the natural environmental disturbance regime. Anthropogenic influence is not, however, random, but guided by cultural continuity, traditions, and tenure. It may therefore influence the morphological variability within *A. tortilis* toward certain growth forms. In the following we will examine combined cultural and natural factors, local use of *A. tortilis* and its management to throw light upon how they may have shaped the distinctive growth-forms of *A. tortilis*.

Method and study area

Acacia tortilis is the dominant element in the perennial vegetation of the Red Sea mountain range in Sudan and Egypt (Fig. 1) and therefore of importance for the local nomadic Beja tribes. The study area reflects a general aridity gradient from the hyper-arid Egyptian region to the less arid Sudanese region [25,26]. Throughout the area there are local variations in aridity within sub-regions caused by a considerable altitudinal gradient and redistribution of water within catchments through an extensive system of dry river valleys (in Egypt/Sudan called wadis/khors). For further description of the study area and the ecology of *A. tortilis* see [2,18,27,28] and the references given there.

The local tribal strategies to manage tree resources have been studied in this area over several decades, through unstructured interviews and participant observations as part of ethno-botanical and ecological field campaigns. In addition, successive field studies have mapped and measured individual trees [2,18,29]. During dedicated field campaigns in 2003 a total of 520 individuals at 33 sites, selected within and among several drainage systems, was morphologically described using the descriptive characters of regional and national floras [28]. Based on these data sets we describe the management strategies that can have a significant effect on tree growth form, and present hypotheses about how the combined effect of these strategies and environmental factors can cause the continuum of tree architecture and growth forms that are observed in the area. The deductive approach was selected because *A. tortilis* growth is extremely slow in the arid core of its distribution area [27], and experiments cannot be done in a feasible timeframe.

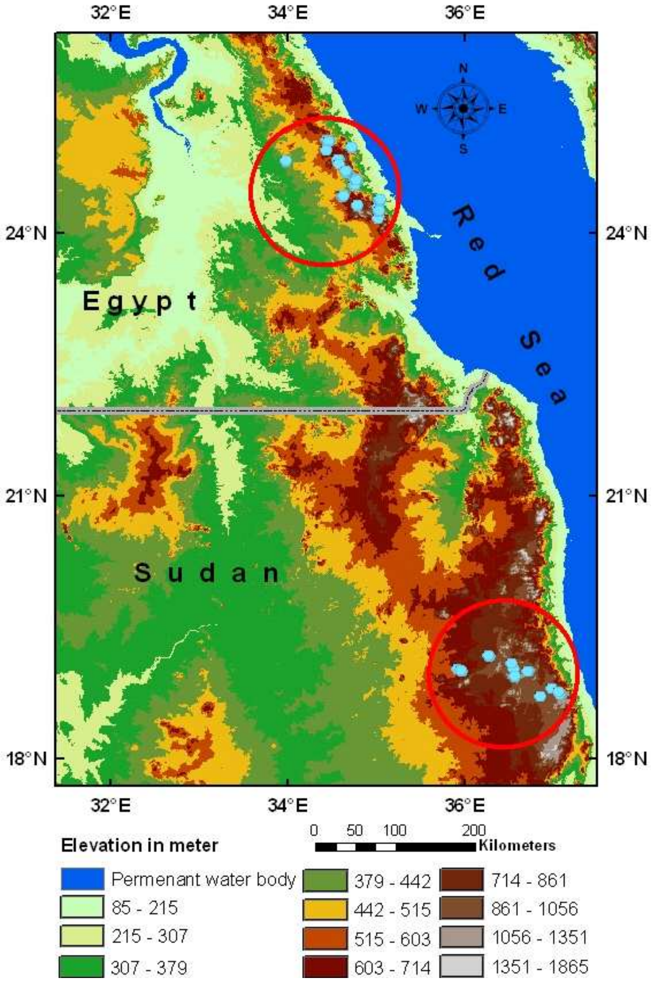

Fig. 1. Study area. Map based on digital elevation model showing the various localities studied (blue dots) within the two regions in Egypt and Sudan (red circles).

Traditional tribal tenure

In arid areas pastoral nomads and their animals depend mainly upon the available trees when and where no other recourses are available. Through millennia local inhabitants have developed and, through cultural continuity, maintained usufruct rights, tenure and management techniques that conserve and sustain their tree resource base (Fig. 2).

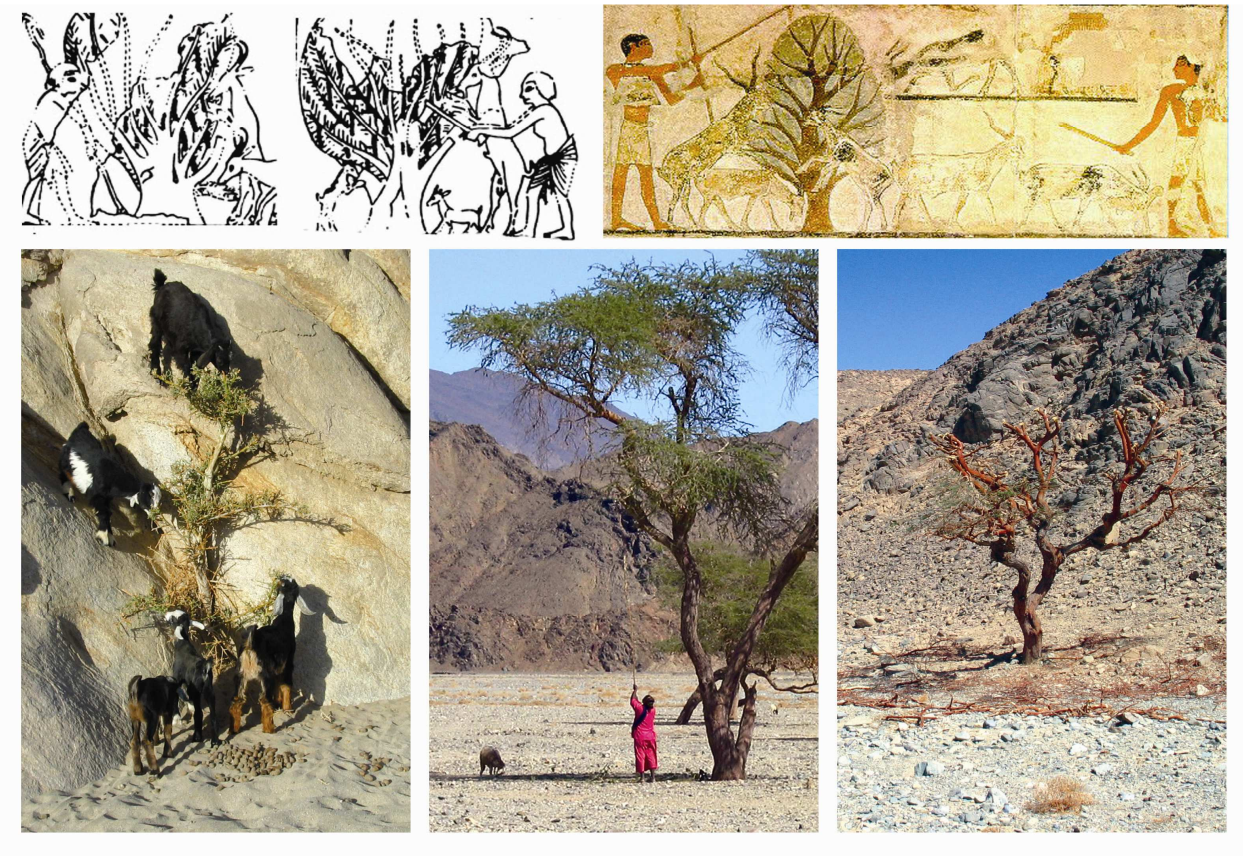

Fig. 2. Traditional nomadic management strategies (direct browsing, shaking branches and pollarding), as depicted already in the Egyptian New Kingdom (upper panel). Modern inhabitants still use the trees the same way (lower panel, photos taken 2003).

One of the primary concerns is a continuous fodder supply for animals. **Browsing** of *A. tortilis* occurs in all stages of its lifecycle, both by wild herbivores such as rodents, gazelles and other ungulates, as well as by domestic animals. Domestic animals do not roam freely; instead goats, sheep, camels and to a small extent cattle are traditionally herded. The pattern of goat and sheep herding varies from place to place according to local traditions and continuity in tribal herding patterns. Traditional nomadic seasonal movement over large distances is presently decreasing, and the typical rapid daily movement at a distance from the camp is increasingly being replaced by practices where the animals roam freely around the campsite. Change in both seasonal and daily herding practices influences the type and intensity of browsing.

Generally *A. tortilis* plants are affected by direct browsing by ovicaprines and camels in their seedling, sapling and bush stages, while large trees are browsed mainly by camels (Fig. 3). On mature trees, camels browse lower branches and margins of the crown. Goats and sheep browse mainly lower, reachable branches, though goats occasionally also climb small shrubs and trees to browse higher branches. When the green biomass is unreachable for the small stock, the herder provides fodder by **shaking crown branches** with a crook (Fig. 2). This supplies animals with small twigs, leaves and pods that fall to the ground.

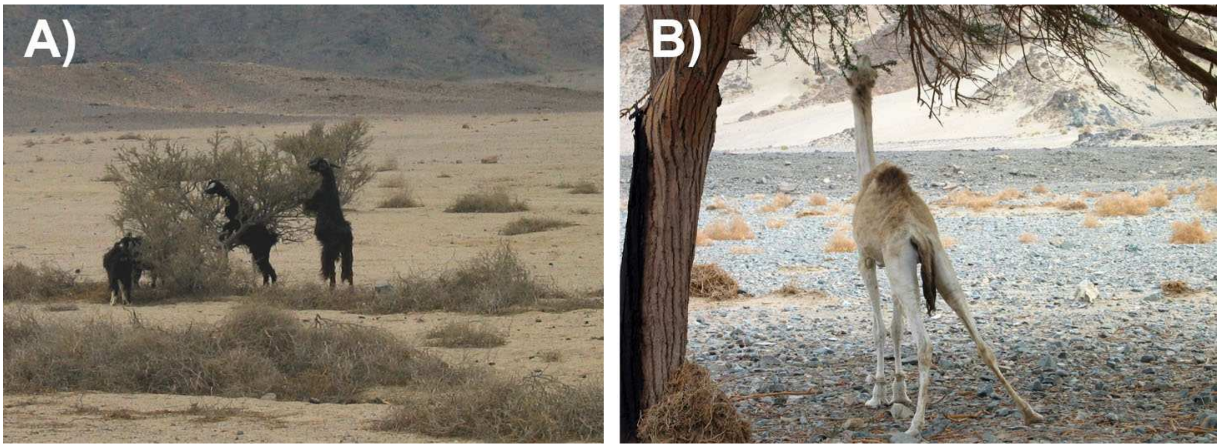

Fig. 3: Browsing by goats (A) and camels (B) form the shape of bushes and trees. The reach of camels typically decides the lower height of the crown.

When and where resources are scarce, fodder from unreachable top-branches is too valuable to be left unexploited. From time to time they are bent down for the night with a rope by travelling camel men to provide fodder, but also to secure that the camel does not wander away in search of fodder during night. Another old, common and widespread management strategy applied to utilize this fodder resource is **pollarding** (Fig. 2), but its importance is not frequently considered in connection with human resource utilization in arid areas. In particular those branches that are unreachable for animals or difficult to shake by the herder’s crook are harvested and left on the ground to provide leaf fodder for the animals. Pollarding is also used as management to remove dry branches. This reduces the transpiration rate and is, according to Beja in Sudan, used actively to prevent the drying out of withering trees [2]. Pollarding over time shapes the trees and improves their vitality and vigor [30]. Along with shaking it is also probably advantageous for reducing/removing infections caused by wax scales [29] and other parasites. As a secondary effect of pollarding, dried branches left on the ground constitute a stable fuel resource for local inhabitants.

The wood of *Acacia tortilis* is highly valued both as **firewood** and for making **charcoal** due to its high fuel quality [30,31], but also as **material for tools and housing**. While the former traditionally is based on dry wood, the latter uses fresh wood and, depending on the purpose of the required material, different life-stages and/or parts of the individual tree are preferred. Due to browsing and pollarding, branches in the crown are typically curved and crooked and only useful for handcraft and minor carpentry. Longer straight material required for tent poles, beds, shanks and walking sticks etc. is often taken from multiple stemmed, smaller trees. The trunk or branch dimension has to be carefully selected according to the requirements set by its final purpose and by available tools. Thick stems are of less use because tools required for reworking are often not available.

Growth forms related to natural and cultural influences

The shaping of *A. tortilis* by natural and anthropogenic environmental factors starts already in its early life stages and continues throughout the life of the tree. In the seedling and sapling stages individuals are very susceptible to disturbances such as browsing and droughts that kill off green biomass [19,21,22,32,33]. In coping with droughts, leaves are shed to reduce water loss by transpiration, and depending on duration, shoots or even whole branches will die back. The effect of droughts on the green biomass in this way resembles that caused by browsing.

While seedlings rarely survive longer droughts or heavy browsing, saplings have a better chance to survive due to their deeper roots. Under better moisture conditions resources are allocated to sprouts and make new shoots. Ability to resprout increases the persistence of saplings [18,23] (Fig. 4). Depending on browsing pressure and/or water availability, dieback and resprouting from the basal part may occur multiple times. In this way growth is suppressed and saplings may remain in that state for several decades [33]. Saplings growing under experimental conditions, protected from browsing and with good soil moisture conditions, as for instance observed in the nurseries in Sinai, typically develop few shoots from the ground [34] (Fig. 4). This is contrary to what happens to saplings growing under unprotected conditions; then they are characterised by dry branches, (remains of) dead main shoots and often with new shoots appearing from the base.

Fig. 4: Resprouting increases the persistence of individuals and is important in all life stages. The combination of drought/soil moisture conditions and browsing influences the degree of resprouting and bushiness of individuals. Even when the main above-ground parts of individuals are gone, stumps may be the basis for new clones (C). Under experimental conditions (D) saplings typically develop few shoots from the base.

Successive resprouting events eventually lead to a sapling with several co-dominant shoots. If only one shoot escapes the combined drought and browsing pressure, a single-stemmed tree may eventually result, but more often, due to the browsing regime, a shrubby or multi-stemmed growth form develops and is maintained [9,32]. The specific effect of browsing depends on the size of the tree as well as on the browser animal and browsing intensity/pressure. High browsing pressure on adult trees leaves trunks bare of branches at lower heights (Fig 5).

The effects of droughts are site-specific, e.g. more severe at locations with thinner soils, steeper slopes or longer distances from a main watercourse. The probability of developing into a multi-stemmed individual (i.e. *A. tortilis ssp. tortilis* or *A. tortilis* ssp. *spirocarpa)* seems therefore to be greater at more drought-prone locations. This idea is supported by the reported variation in drought resistance for the various growth forms; in order of decreasing tolerance: *A. tortilis ssp. tortilis, A. tortilis* ssp. *spirocarpa and A. tortilis* ssp. *raddiana* [9,12,35]. The single-stemmed form (i.e. "*A. tortilis* ssp. *raddiana*") is predominant at sites with better soil moisture; but, since this pattern depends on local moisture conditions, it does not conflict with the abundance of "*A. tortilis* ssp. *raddiana*" in hyper-arid deserts such as the Eastern Desert of Egypt and the Negev, Israel.

As saplings become well established/deeply rooted, i.e. more drought resistant, and grow into larger individuals, it is mainly browsing and eventual pollarding and collection of material from them that will shape their architecture. The diversity in the utilisation of trees results in a multiplicity of growth forms. The distinction described between the multi-stemmed, flat-topped large individuals, on the one hand, and the single-stemmed trees with round/irregular crowns, on the other, is not supported [5,13,16]. Rather the whole spectrum of intermediate forms can be observed (Fig. 5).

Fig. 5: Morphological diversity is great in *Acacia tortilis*, there is no clear distinction between growth forms, and the whole spectrum of intermediate forms can be observed (I). The lower panels (II and III) show the effect of pollarding on the shape of the crown and the effect of material collection (removal of main stems). Note that IIIB is manipulated (cf. IIIA) in order to show the flattening of crowns by pollarding branches out of reach for browsing animals. It is characteristic for all managed types that at lower heights the trunk has no branches due to the browsing pressure.

When several co-dominant, upward-growing trunks, branches and branchlets have to compete for available space, a wide growth form will result. This gives multi-stemmed trees a flat-topped appearance, but the spread of stems also creates an optical flattening effect: a crown of a certain height will look flatter on a wide growth form than on a single-stemmed individual which has a less spread crown. Added to this, browsing and/or pollarding affect the crown shape. Smaller trees are browsed from above by camels and from below by goats and sheep, reinforcing the flat crown type. For taller trees there is browsing pressure only from below, mainly by camels. The animals reach the lower branches and margins of the crown. The resulting lower crown level is determined by their reach (about 270 cm; cf. Fig. 3) [32]. Internal and upper parts of the crown are protected from browsing and top branches may therefore develop faster growth. When this fodder resource is harvested by pollarding, the crown is again flattened (Fig 5).

Sometimes the crown of a tree may be heavily pollarded, leaving only lower parts of its main branches (Fig 5). New shoots growing from the branch-stumps are numerous and have a bushy appearance. Depending on the shape of the remaining branches, an irregular or dense rounded crown will eventually form. When branches grow out of reach to browsing, pollarding of top branches together with browsing from below will again lead to flattening of the crown. Consequently, the crown shape varies over time and depends on the degree and cycle of pollarding and on browsing pressure. This contributes to the wide variety of crown shapes observed in field (Fig. 5).

The number of main trunks is, with time, affected by the collection and use of straight wood material for constructions etc (cf. above). Wood of predetermined dimensions is preferred for certain products; e.g. thinner branches are selected for walking sticks than for tent poles. Anyhow, the effect is a human-induced selection towards few- or single-stemmed mature trees in managed systems. Because trees are of great importance for local nomads, at least one trunk will be preserved.

With increasing tenure and management pressure, material collection favours a development towards fewer main trunks, while pollarding tends to produce a more rounded/irregular crown. When this happens, the growth form corresponding to "*A. tortilis* ssp. *raddiana"* can be interpreted as a result of the most intense management regime. "*A. tortilis* ssp. *raddiana"* is the ecotype distributed in the extreme core of arid environments where there is a high utilisation pressure because of limited resource availability.

Unusual growth forms, combining a typical "*A. tortilis* ssp. *raddiana"* form with a bushy "*A. tortilis* ssp. *tortilis"* form at lower reaches, are sometimes observed (Fig 6). Sometimes even mature trees are induced to resprout from the base or the trunk itself. This has been observed on trees partly destroyed by charcoal producers. If browsing pressure is low, as it is in areas where nomads settle, become dependent on charcoal production, or change their herding strategies, trees may grow into a shrubby appearance at lower reaches while there is a more typical crown higher up. The potential of mature trees to resprout from the base of the main trunk or even from roots is particularly important for vegetative reproduction in areas where recruitment from seeds is lacking or extremely rare [18]. Through such vegetative reproduction, the new clone will have a better chance for survival by taking advantage of its extensive root system, and dominant shoots may escape browsing and/or drought suppression more easily. Large single-stemmed individuals can, therefore, be resprouters, not reseeders as suggested for acacias in other areas [22; but see 23].

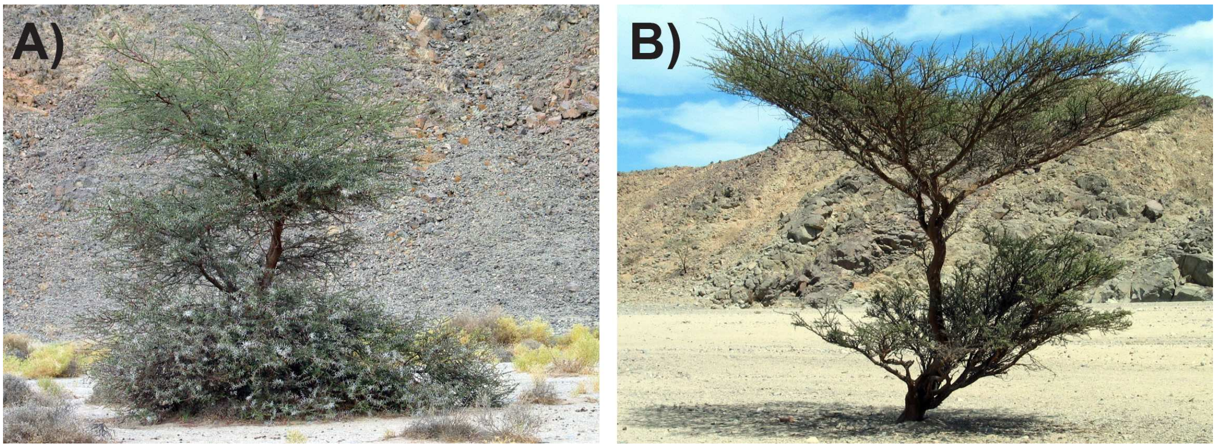

Fig 6: Unusual growth forms, combining a typical "*A. tortilis* ssp. *raddiana"* form with a bushy "*A. tortilis* ssp. *tortilis"* form at lower reaches, are also observed.

Conclusions

Growth-form (bush, multi-stemmed or single-stemmed trees), and shape of the crown (diffuse, rounded, flat, spreading), have been main characters used to separate the former *A. tortilis* complex into subtaxa. As the present taxonomy of the *A. tortilis* complex is not genetically supported, its morphological diversity must be attributed to environmental factors including natural as well as cultural pressures. While disturbances due to drought and browsing by wild herbivores are stochastic events that will result in a range of growth forms, they will, in combination with human tenure and animal herding, form specific growth architectures and appearances.

The observed morphological variability within *Acacia tortilis* may therefore be a result of ecological conditions in combination with local, traditional use in the different life-stages of the trees. We argue that bush-shaped and multi-stemmed shorter trees with "*A. tortilis* ssp. *tortilis"* and *"A. tortilis* ssp. *spirocarpa*" morphology are the result of combined browsing and drought pressure in their sapling and bush stages, while single-stemmed trees with rounded/irregular canopies, i.e. the growth form corresponding to "*A. tortilis ssp. raddiana",* are a result of an intense management regime in a later life stage.

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