

MORPHOLOGICAL STUDIES OF THE SUBGENUS *SERIPHIDIUM* (BESSER) ROUY OF *ARTEMISIA* L. IN BALOCHISTAN (PAKISTAN)

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Abstract

Based on 2,500 herbarium vouchers of the subgenus *Seriphidium* of *Artemisia*, which were collected from 21 sites across Central and NW-Balochistan, morphological studies and detailed drawings of stem leaves were performed with the aim to be able to identify the most common *Artemisia* species of Balochistan by a few vegetative characters. The following species were recognized: *Artemisia quettensis* (most common and endemic species in the area), *A. turanica*, *A. oliveriana*, *A. sieberi*, *A. stenocephala*, and *A. santolina*. Some presumed hybrid species had intermediate morphological characters, such as *A. quettensis* x *A. oliveriana*, *A. quettensis* x *A. turanica*, *A. oliveriana* x *A. turanica*, and *A. oliveriana* x *A. sieberi*. Key characters of the species, illustrations, and data on the distribution were provided. The frequent polyploidization and hybridization processes within this species complex were emphasized, and the effects on speciation and habitat preference were discussed. The present study also suggests a possible relationship between species distribution patterns, xeromorphism and climatic factors, varying from northeast to southwest Balochistan.

Key words: *Artemisia*, Morphology, Identification key, Balochistan (Pakistan).

Introduction

The uplands of Balochistan are traditional old rangeland and natural vegetation has been degraded and transformed into a widespread mixed grass-shrub steppe, including various *Artemisia* taxa (Rafi, 1965; Khan, 1991; Akhter & Mirza, 2006; Mirza *et al.*, 2009). However, taxonomy, nomenclature, as well as the distribution range of the subgenus *Seriphidium* of *Artemisia* from Central Asian steppes are still and partly controversially discussed among several authors. This may be due to high levels of within-species phenotypic plasticity in combination with probably frequent polyploidization and hybridization processes within this species complex (Podlech in Rechinger, 1986, pp. 159-161). Several studies used thus general terms like “*Artemisia*-species” as Rodriguez (1994), and Ahmad and Yasmin (2011) or “*Artemisia maritima*” (e.g. Troll, 1939; Chaudhri, 1957, 1960; Stewart, 1961; Webster & Nasir, 1965; Hartmann, 1968; Tareen & Qadir, 1987, 1990, 1991; Sultani *et al.*, 1993; Rhind, 2010; Badshah *et al.*, 2016). However, the latter term exists for a multitude of lower taxonomic levels as it was already stated by Hooker (1882, pp. 323-324) and Pampanini (1927), and more recently by Kaul and Bakshi (1984) and Kumar *et al.*, (2011). For instance, *A. stenocephala*, *A. quettensis*, *A. brevifolia*, and *A. sieberi*, which all belong to the flora of Balochistan, have been reported as synonyms of *A. maritima*, probably because these species were not yet recognized or described at that time. The species *Artemisia maritima* sensu stricto is limited to western and northern European coasts (Meusel *et al.*, 1965; Persson, 1974; Tutin *et al.*, 1976). Another example refers to *Artemisia herba-alba*, which was also frequently indicated for Balochistan (Marwat *et al.*, 1989, 1990; Rabie *et al.*, 2006; Rhind, 2010; Kursat *et al.*, 2011). This species, in fact, is a West Irano-Turanian element, extending until the Atlantic coast in North Africa, but is still missing in Central Asia (Meusel *et al.*,

1965; Tutin *et al.*, 1976). The species has also been regarded as a synonym of *A. sieberi* (Podlech in Rechinger, 1986; www. eFloras.org, Flora of Pakistan). Since some issues on the reliable identification of Central-Asian *Artemisia*-taxa remained open to the related literature of Pakistan and adjacent countries (compare i.e. Dickorè & Nüsser, 2000; Eberhardt, 2004; Peer *et al.*, 2001, 2007), the preoccupation with this taxon has been one of the key arguments in our present study.

Referring to the nomenclature within the high variety of synonyms and homonyms and the changing opinions on the *Artemisia* taxon, we aligned ourselves with Podlech (in Rechinger, 1986, Flora Iranica), who divided the genus *Artemisia* into the three subgenera *Artemisia*, *Dracunculus*, and *Seriphidium*. This is in accordance with the latest phylogenetic findings such as those of Kornkven *et al.*, (1998), Torrell *et al.*, (1999), Watson *et al.*, (2002), Sanz *et al.*, (2008), Oberprieler *et al.*, (2007, 2009), and Malik *et al.*, (2017). There are no arguments to consider subgenus *Seriphidium* an independent genus (compare Kardereit & Jeffrey, 2007; Funk *et al.*, 2009, and Hayat, 2011). Nevertheless, this interpretation is in contrast to the classification used in the Floras of Pakistan and China (Ali & Qaiser, 2002; Wu *et al.*, 2011; www. eFloras.org, Flora of Pakistan; www. eFloras.org, Flora of China), as well as to Bremer and Humphries (1993), based on morphological evidence.

The Old world section *Seriphidium* comprises about 130 taxa native to Europe and temperate Asia, with the largest number of species in Central Asia (Shiskin & Bobrov, 1961; Podlech, 1986; Ling, 1991a, b; Wu *et al.*, 2011). In Pakistan, the section is represented by 13 taxa (Ali & Qaiser, 2002; www. eFloras.org, Flora of Pakistan). In this study, we examined *Artemisia* subgenus *Seriphidium* on the basis of field data and herbarium vouchers from the southern Pakistani region Balochistan aimed to answer the following questions: (i) which

Artemisia species occur in the research area, (ii) how can the most common *Artemisia* species be vegetatively distinguished with the help of particular keys and detailed drawings, and (iii) do habitat differences affect distribution patterns of *Artemisia* populations?

Materials and Methods

Site description: Topographic position and a few terrain features of the sample collection sites were presented in Table 1. Almost all sample sites are located in central Balochistan except of the one site in Saindak (Chagai district, CGI), which is situated in the north-west corner of Balochistan near the Iran border (Fig. 1). The altitude of all sampling sites ranges from 980m to 2,420m above sea level and includes different habitats such as piedmont plains, piedmont fans, quaternary moraine terraces, and large river plains (Scholz, 1974; Changezi, 1982). The area reflects a relatively young geological evolution of still continuing alpine orogeny. In the present time, denudation and depositional processes dominate the scenery. The bedrocks include igneous rocks (ophiolite, ultramafic and mafic rocks, serpentine melange, dolerite), highly fossiliferous limestone, sandstone, mudstone, and quaternary shale/siltstone (Kazami, 1979; Bender & Raza, 1995; Welcomme *et al.*, 2001). The climate is continental influenced, with cold and sometimes snowy winters and hot summers. The precipitation regime is characterized by a gradient that ranges from ≥ 300 mm in the mountainous northern and eastern parts to 150–300 mm in the central parts, and ≤ 100 mm in the south and north-west plains near the Iranian border. To provide more detailed information on the regional rainfall distribution, we extracted the annual precipitation for each collection site from the niche modelling software package WorldClim-climate data (Hijmans *et al.*, 2005). Increasing drought periods, land degradation by overgrazing and fuel collecting are long-standing problems of arid rangelands of Balochistan. Desertification is thus on the increase, as is salinization and soil erosion through wind and water (Hussain & Chughtai, 1984; Islam *et al.*, 2004; Ahmad *et al.*, 2012).

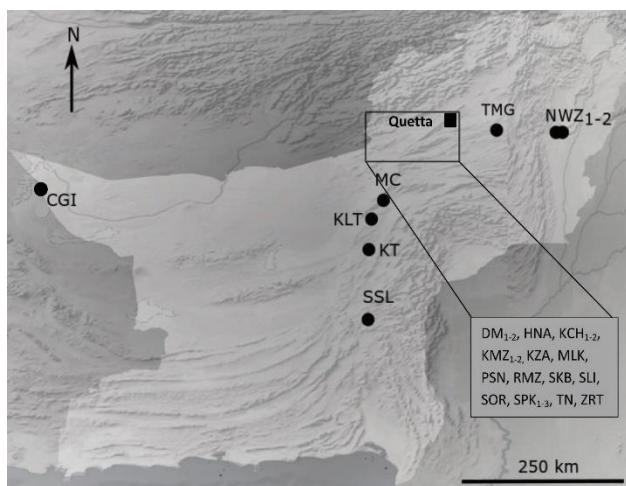


Fig. 1. Map of *Artemisia* sampling sites in central and NW-Balochistan. For population codes see Tab. 1.

Data collection

Under the direction of Dr Rubina Akhter from the National Herbarium Islamabad about 2,500 individuals of *Artemisia* taxa, mainly of the subgenus *Seriphidium*, were collected between April and May 2008. They were distributed over 21 sites, following a northeast to southwest transect. Most sampling sites, however, concentrated around Quetta. Individuals were often gathered from sites close to roads or villages, on river basins, piedmont fans, plains, and on foot slopes (Table 1). In each site, 4 to 12 *Artemisia* populations were randomly selected (219 populations in total) and from each population 3 to 15 individuals were herbarized. Most populations were dominated by only one *Artemisia* species; however, in some sites several *Artemisia* species co-occurred and formed mix populations. A total number of 862 herbarium voucher specimens were arranged and sorted taxonomically, representing now one of the largest collections of the subgenus *Seriphidium* of *Artemisia* from Balochistan in Europe. All vouchers are deposited at the herbarium of the University of Salzburg (SZU), Austria.

Morphological studies: All herbarium specimens were first taxonomically examined with a Zeiss stereomicroscope at 10-40x magnification, following the taxonomic descriptions in Podlech (in Rechinger, 1982), Shishkin and Bobrov (1961), and the Flora of Pakistan (Ali & Qaiser, 2002; www.efloras.org, Flora of Pakistan).

Because of the early collection season in the field, only vegetative individuals and partly incomplete plant components were available. Capitula were not yet developed, and basal leaves were generally withered and crumbled. Exceptions here were some individuals of *A. sieberi*, which we additionally collected in February 2016 in S-Iran. Identified species were cross-checked with the *Seriphidia* collection of Prof. Dieter Podlech from the herbarium of the Botanische Staatssammlung München (M), Germany. Dubious specimens were additionally checked at the herbarium of the Natural History Museum Vienna (W), Austria.

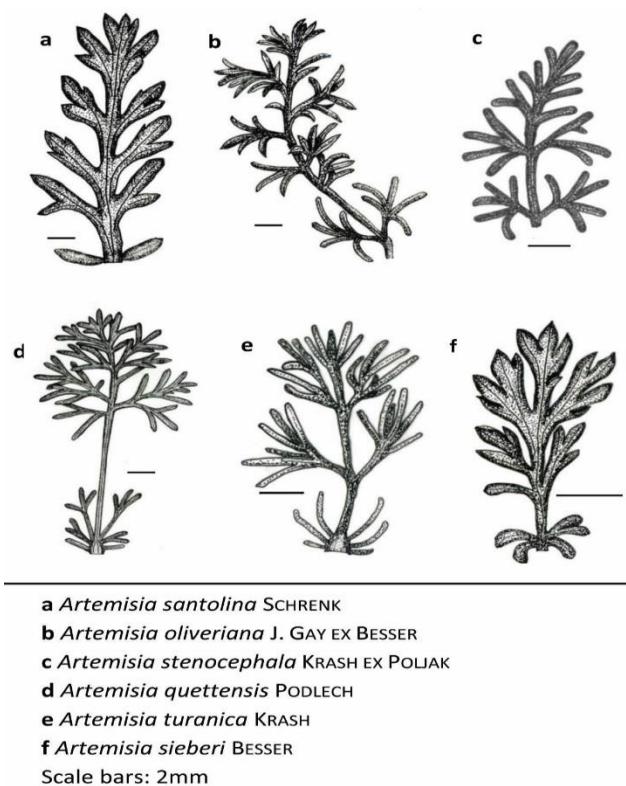
For detailed comparison of size and shape of leaves, 10-15 individuals per species and 5-8 leaves per individual we used in the biometric analysis. Herbarium leaves were first softened by immersing in Deconex solution overnight (to reduce the water surface tension) and then analysed by a specific Leica EZ4D stereomicroscope (10-40x magnification) with integrated LED illumination, Digital 3 MP Camera, and LAS EZ software (vers. 3.1.0). The photographs of the stems were taken with the same microscope. The following morphological variables were assessed from the herbarium specimens: leaf, leaflets, stipules, indumentum, stem, and inflorescence (Table 2).

Table 1. Location and habitat characteristics of the sampling sites for *Artemisia* populations in central and NW-Balochistan. Site specific ecological characteristics were recorded during sampling and/or extracted from Google maps data, based on the geographical coordinates. Coordinates in degrees and minutes.

Population	Number of populations	Location and location code	District	Longitude	Latitude	Altitude [m a.s.l.]	Annual precipitation [mm]	Habitat
<i>A. queettensis</i>	4	Hanna lake HNA	Quetta	67.05	30.15	1910	251,41	stony piedmont plain river basin, silty fine sediment
<i>A. queettensis</i>	10	Kalat KLT	Kalat	66.35	29.02	2012	164,07	rocky slope
<i>A. queettensis</i>	10	Kalat SKT	Kalat	66.31	28.58	2080	167,26	field edges, stony piedmont fan
<i>A. queettensis</i>	3	Kan Methar Zai KMZ ₁	Pishin	67.28	30.43	2200	277,01	field edges, stony piedmont fan
<i>A. queettensis</i>	3	Kan Methar Zai KMZ ₂ , near KMZ ₁	Pishin	67.27	30.42	2180	276,63	field edges, stony piedmont fan
<i>A. queettensis</i>	10	Karkhasa park KZA	Quetta	66.56	30.10	1830	223,56	stony scree slope
<i>A. queettensis</i>	10	Maslakh Range MLK	Pishin	66.46	30.12	1660	237,65	plains, silty fine sediment
<i>A. queettensis</i>	5	Nala Wali Zai NWZ ₁	Loralai	69.11	30.27	1260	265,48	river basin, stream bed
<i>A. queettensis</i>	5	Nala Wali Zai NWZ ₂ , near NWZ ₁	Loralai	69.11	30.27	1270	265,48	piedmont plain
<i>A. japonica</i>	10	Pishin, road side PSN	Pishin	66.59	30.35	1540	255,99	river basin, sandy, gravelly sediment
<i>A. queettensis</i>	10	Road Mula Zai RMZ	Quetta	67.22	30.37	2200	272,13	hilly piedmont
<i>A. queettensis</i>	10	Surkhab Karez SKB	Pishin	67.04	30.35	1540	262,38	low hills
<i>A. queettensis</i>	10	Samungli SLI	Quetta	66.55	30.14	1600	247,87	piedmont fan
<i>A. queettensis</i>	10	Sor, range-Degari, coal mines SOR	Quetta	67.12	30.09	2130	254,05	ridge slope
<i>A. queettensis</i>	10	Sasol SSL	Khuzdar	66.40	27.52	1400	164,24	piedmont fan, fine sediment and stones
<i>A. queettensis</i>	10	Kanak valley, Toot Nala TN	Mastung ^g	66.48	30.03	1800	222,37	foot slope
<i>A. queettensis</i>	10	Ziarat ZRT	Ziarat	67.42	30.23	2420	285,32	scree slope, valley plain
<i>A. queettensis</i>	10	Kanak valley, dam site DM ₁	Mastung	66.49	30.06	1900	228,32	stony-silky piedmont fan
<i>A. turanica</i>	10	Kanak valley, dam site, DM ₂ , near DM ₁	Mastung	66.49	30.06	1900	228,32	stony-silky piedmont fan
<i>A. queettensis</i>	7	Mangochar MC	Kalat	66.40	29.26	1890	137,63	piedmont fan
<i>A. turanica</i>	4	Mangochar MC						
<i>A. queett. x A. tur.</i>	1							
<i>A. queettensis</i>	6							
<i>A. queett. x A. oliv.</i>	1	Spin Karez, around lake SPK ₁	Quetta	67.08	30.13	2000	252,32	river basin
<i>A. oliv. x A. tur.</i>	1							
<i>A. oliveriana</i>	1	Spin Karez, around lake SPK ₂	Quetta	67.08	30.13	2000	252,32	river basin
<i>A. oliv. x A. tur.</i>	3							
<i>A. turanica</i>	5	Spin Karez, around lake SPK ₃	Quetta	67.12	30.15	2000	252,32	river basin
<i>A. queett. x A. tur.</i>	3							
<i>A. queettensis</i>	7	Kach KCH ₁	Ziarat	67.19	30.26	1910	265,57	river basin
<i>A. sanctolina</i>	1	Kach, KSH ₂ , near KCH ₁	Ziarat	67.19	30.26	1910	265,57	river basin
<i>A. stenocaphala</i>	2							
<i>A. sieberi</i>	6	Saindak Chaghi CGI	Chagai	61.36	29.13	980	73,72	sandy piedmont plain
<i>A. oliv. x A. sieberi</i>	1	Saindak Chaghi CGI	Chagai	61.36	29.13	980	73,72	sandy piedmont plain
<i>A. oliveriana</i>	9	Asghar, Tomagh TMG	Ziarat	68.08	30.18	1920	265,65	ridge, slope
<i>A. queettensis</i>	1							
Total	219	21						

Table 2. Diagnostic key characters of the six *Artemisia* species.

	<i>A. quettensis</i>	<i>A. turanica</i>	<i>A. oliveriana</i>	<i>A. stenocephala</i>	<i>A. sieberi</i>	<i>A. santolina</i>
Stem	slightly ribbed, reddish-brown, peeling bark; less hairy	purple/black- violet-brown, glossy glabrous to slight hirsute	markedly ribbed, yellowish-brown; arachnoid-tomentose	markedly ribbed light brown densely tomentose	yellowish-brown, peeling bark, abundantly short hairy	light brown, peeling bark less to strongly hairy
Lamina length (mm)	6-17	8-16	12-22	10-15	8-16	12-16
Lamina width (mm)	6-11	7-15	6-10	8-12	6-15	6-7
Petiole length	long petiolate 30-50 mm	short petiolate 5-10 mm	long petiolate 30-60 mm, sometimes shorter	short petiolate (5-10 mm), sometimes longer	short petiolate 5-15 mm	sessile
Shape of lamina/ median stem leafs	orbicular-ovate, deep lobed	orbicular-ovate, pinnate, funnel-like	ovate-oblong, falcate/ curved, long lasting	subobtuse, all leaves withering early	ovate, deep lobed	ovate, weak lobed to simple
Lobes/leaflets	broadly decurrent; deeply pinnatisect; terminal lobes obovate; obtuse; ribbed along leaf veins; cross section flatly	almost not decurrent; funnel-like; deeply pinnatisect; terminal lobes linear; acute; cross section rounded	narrowly decurrent; deeply pinnatisect; terminal lobes lanceolate; mucronate to obtuse; falcate; cross section flatly	terminal lobes subobtuse to obtuse; sometimes slightly falcate and mucronate; cross section flatly	broadly decurrent; weakly pinnatisect; terminal lobes oblong; acute; cross section flatly	broadly decurrent; weakly pinnatisect; terminal lobes broadly; mucronate; cross-section flatly
Leaf sheath	broad, concaulcent	broad, swollen, concaulescent			± absent	nearly absent
Stipules	long petiolate; pinnate	long linear; emerge at the swollen leaf sheath	petiolate, pinnatisect; curved/ falcate	short petiolate, pinnatisect	sessile to short petiolate; pinnatisect	sessile, simple, oblong
Indumentum/ middle leaves	arachnoid dense tomentose	tomentose	tomentose	tomentose	canescent-arachnoid	tomentose
Single hairs/ middle leaves	filiform and glandular	filiform, stellate and glandular	not analysed	filiform, stellate and glandular	filiform and glandular	
Inflorescence	slender	broadly conical	broadly pyramidate	broadly pyramidate	broadly conical	broadly expanding
Branches of inflorescence	erect-adpressed, short ascending	horizontally extended, flexuously	ascending, erect	spreading, robustly	horizontally spreading, almost spiny	horizontally extended, robustly

Fig. 2. Comparison of typical stem leaves of the six *Artemisia* species.

Results

Species diversity: Based on growth form and morphological characters as proposed in the available taxonomic literature, we identified most of all examined vouchers as *A. quettensis* Podlech (70, 5%) which is considered as an endemic species in SW-Pakistan. The holotype, which was taken for comparison, comes from Spin Karez (district Quetta) and is stored at the herbarium of the Botanische Staatssammlung München (M), Germany. The other identifiable *Artemisia* taxa include the species *A. turanica* Krash. (9%), *A. oliveriana* J. Gay ex Besser (5%), *A. sieberi* Besser (1,5%), and *A. stenocephala* Krash. ex Poljak. (ca. 1%). A few populations of *A. santolina* Schrenk (ca. 0,2%) we could detect in Kach (Ziarat district). This species is not listed in the flora of Pakistan and probably new to Pakistan. About 4% of all populations were classified as putative hybrids, several being still of questionable validity. The species *A. japonica* Thumb. from Pishin belongs to the subgenus *Dracunculus* and is therefore not relevant to the present paper. About 8% of the whole collection could not be determined at least due to incomplete characteristics and characteristics which were not in accordance with the taxonomic identification keys of the available floras. The species *A. freitagii* Podlech we couldn't find in the study area, although reported for Balochistan. The morphological characters are in any case such significant so that this species could have not been overlooked. The species seems to be rare (endemic to Pakistan) and restricted to higher regions (1,800-2,300m). The differentiation between *A. oliveriana* and *A. stenocephala*, however, proved to be difficult due to similar traits. Even Podlech (in Rechinger, 1986) reported possible confusions between the two species. The description in the Flora Iranica in the Latin language is not such as to clearly differentiate the two *Artemisia* species.

Diagnostic key characters of the six *Artemisia* species are listed in Table 2. Drawings of stem leaves, incl. leaf successions are illustrated in Figs. 2-7. *A. stenocephala*, however, could not be depicted because of rarity, incomplete leaf succession, and early crumbling leaves. Stipules, which we found on the ground of all stem leaves, are particularly remarkable because they have not yet been mentioned in the relevant floras. As is shown in Fig. 8, the younger stems differ in structure, colour, and indumentum.

Despite the high morphological variation and transition forms within and between all examined *Artemisia* species, a useful tool for a better differentiation of the six *Artemisia* species is now being offered for the first time by the given key-characters and drawings.

Putative hybrids were mainly found in sites where different populations co-occurred (MC, SPK, CGI). They are most frequent in the combination of *A. oliveriana* and *A. turanica*. Curved lobes and narrowly decurrent leaflets are compliant with *A. oliveriana*. Also the filiform and glandular single hairs tend to *A. oliveriana*. On the other hand, purple coloured young stems and the inflorescences with horizontally extended and flexuously branches point to *A. turanica*. Both species are common to central Balochistan, and have similar distribution areas. *A. oliveriana* may also hybridize with *A. quettensis*. Long petioles and intermediate leaf size may point to *A. quettensis*. Lobes are broadly decurrent. A third mixed form was found between *A. quettensis* and *A. turanica*, which, however, is a widespread species in drier parts of central Asia and central Balochistan. Such specimens have long petiole and broadly decurrent leaflets similar to *A. quettensis*. Moreover, purple young stems and a broad inflorescence with ascending branches may hint at *A. turanica*. Finally, in *A. oliveriana* x *A. sieberi* lobes are rather flat and slightly curved. Stem is angular formed, slightly peeling, and the panicle has horizontally lateral branches on the other hand.

Geographical distribution and xeromorphism: Distribution maps were compiled for the species *A. quettensis*, *A. stenocephala*, *A. oliveriana*, *A. turanica* (incl. *eremophila*), *A. santolina*, and *A. sieberi* (incl. *dumosa*), following the specifications in Shishkin and Bobrov (1961), Podlech (1986, 2013), Ling (1991a, b), and Wu *et al.*, (2011). As shown in Fig. 9, *A. santolina* is widely distributed, ranging from Balochistan (near Kach/Ziarat, perhaps a new record) to Iran and to the deserts of Kazakhstan, Uzbekistan, Turkmenistan, and NW-China (Xinjiang). So far, there is no record in Afghanistan (Breckle, 2012). *A. turanica* is also a wide-ranging taxon and occurs in SW-Pakistan (Balochistan), Afghanistan, SE-Iran, as well as in dry parts of Turkmenistan, Uzbekistan, Kazakhstan, Tadzhikistan, and Kirgizstan. *A. oliveriana* is common to central Asia (Pakistan, Afghanistan, Iran, Uzbekistan, Kazakhstan, Turkmenistan) and has a new record in SE-Turkey (Firat, 2015). *A. sieberi* extends over central Asia (SW-Pakistan, Afghanistan, Turkmenistan, Iran, Iraq) to Syria, Palestine and Anatolia (Turkey) in the west. The subendemic *A. stenocephala* is distributed over SW-Pakistan and Afghanistan, including an occurrence in the mountains of Koped-Dag (Turkmenistan). Finally, the endemic *A. quettensis* is geographically restricted to Balochistan with a small overlap in NE-Iran (provinces Sistan and Baluchestan, see Podlech in Rechinger, 1986). This species is also not indicated in Afghanistan (Breckle, 2012).

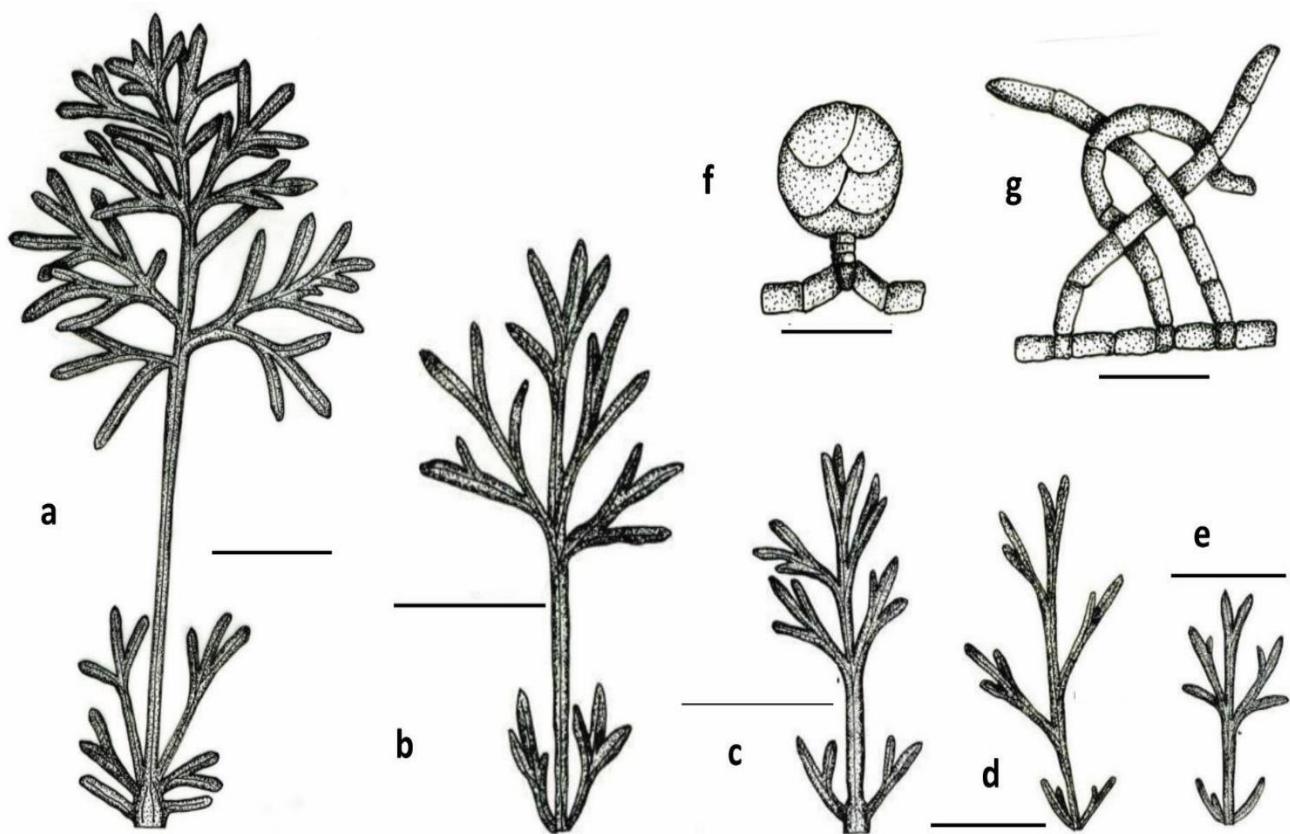


Fig. 3. *Artemisia quettensis* PODLECH. Leaf sequence. a lower stem leaf, b middle stem leaf, c upper stem leaf, d stem leaf in inflorescence area, e bract, f glandular trichome, g simple, uniseriate trichomes. Scale bars: a: 10 mm, b-e: 2mm, f-g: 50 μ

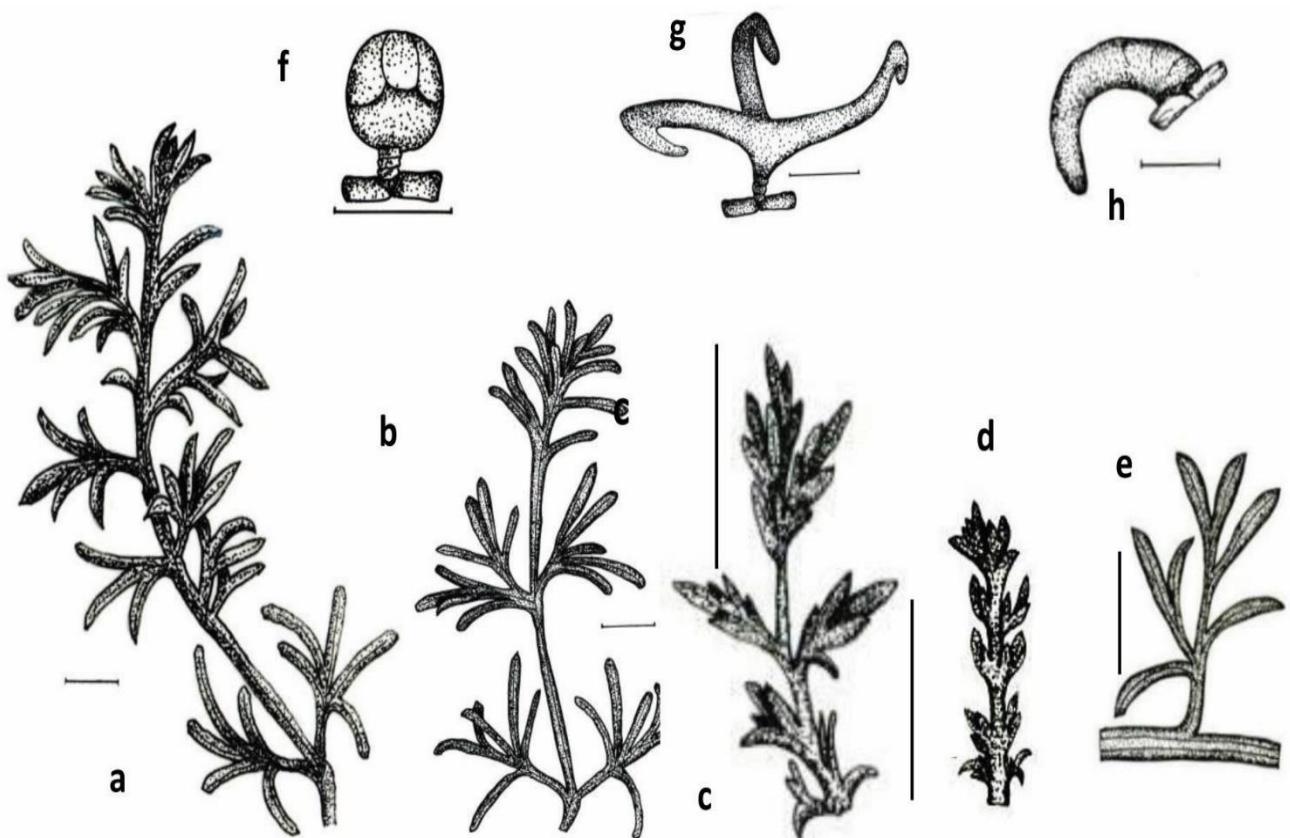


Fig. 4. *Artemisia oliveriana* J. GAY EX BESSER: Leaf sequence. a lower stem leaf, b middle stem leaf, c upper stem leaf, d leaf in inflorescence area, e leaflet of stem leaf, f glandular trichome, g stellate trichome, h curved singular trichome. Scale bars: a-e: 2mm; f-h: 50 μ .

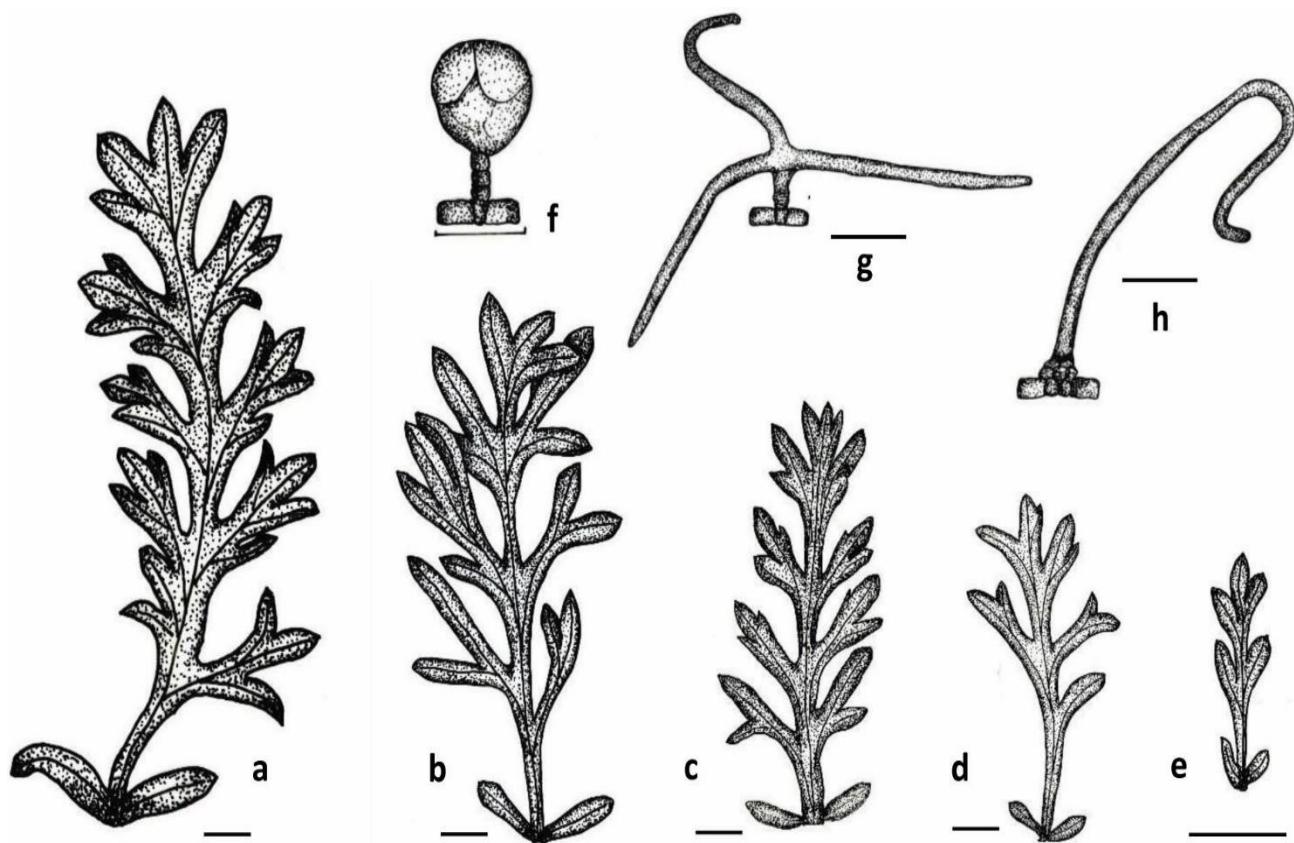


Fig. 5. *Artemisia santolina* SCHRENK. Leaf sequence. a lower stem leaf, b middle stem leaf, c upper stem leaf, d leaf in inflorescence area, e bract, f glandular trichome, g 3-rayed, short- stalked stellate trichome, h simple, uniseriate trichome. Scale bars a-e: 2mm, f-h: 50 μ .

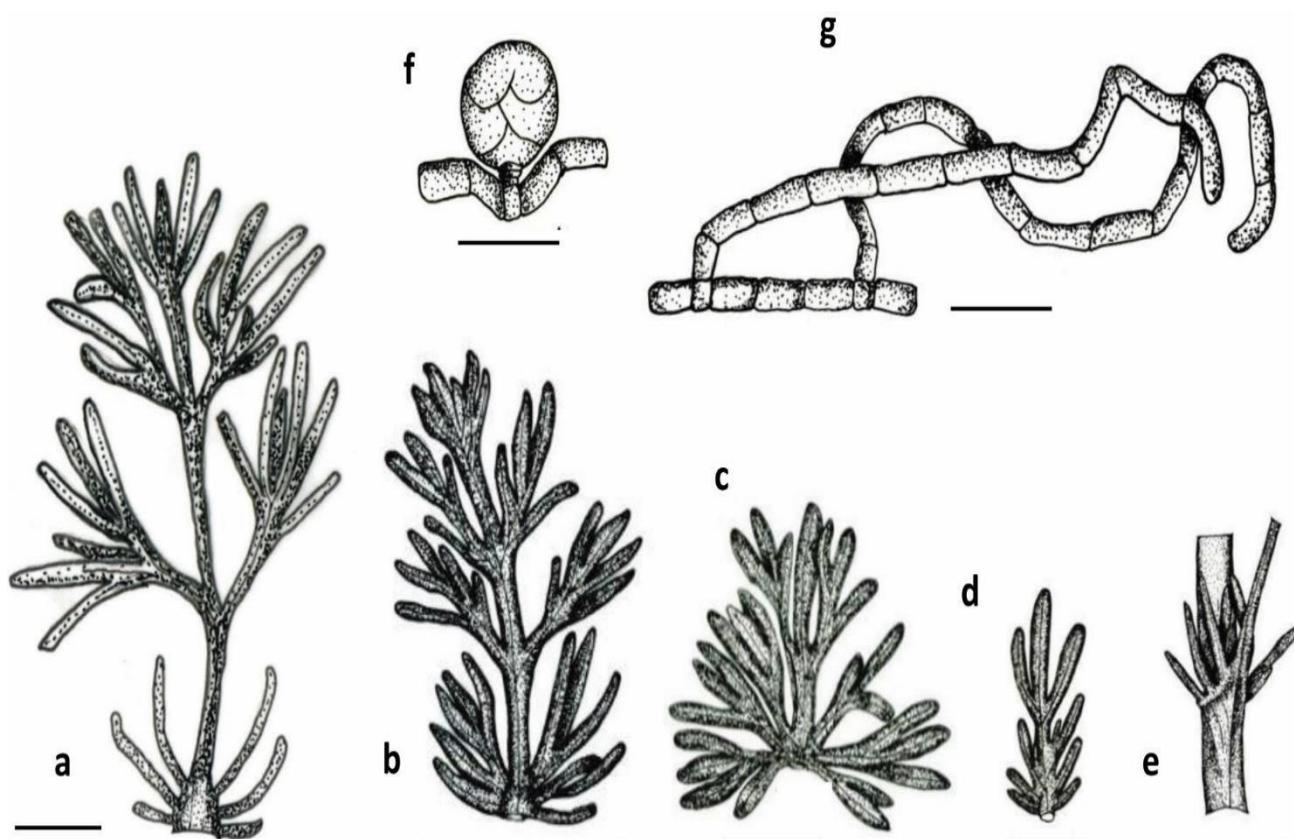


Fig. 6. *Artemisia turanica* KRASH.: Leaf sequence. a lower stem leaf, b middle stem leaf, c upper stem leaf, d stem leaf in inflorescence area, e sheath of a basal leaf, f glandular trichome, g simple, uniseriate trichomes. Scale bars: a-d: 2mm, e: 1mm, f-g: 50 μ .

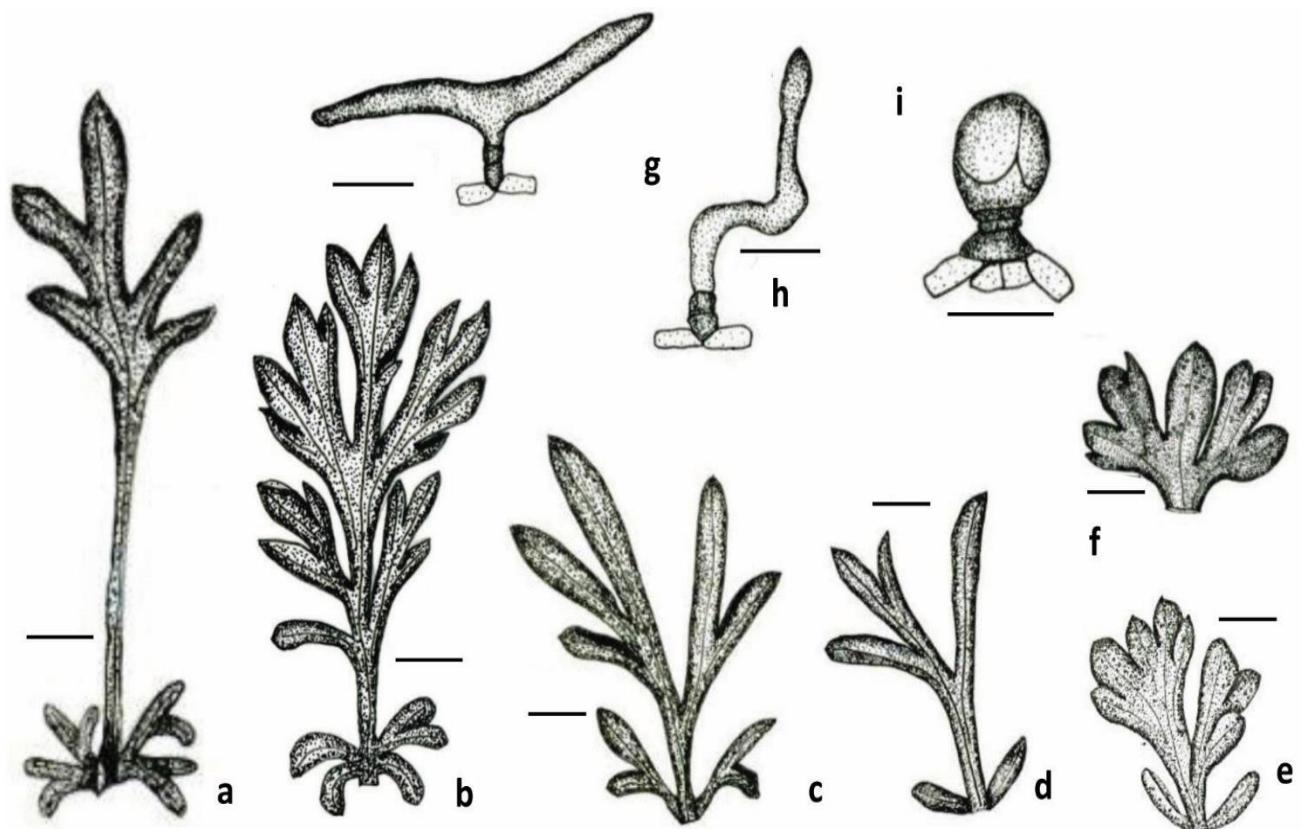


Fig. 7. *Artemisia sieberi* BESSER. Leaf sequence. a basal stem leaf, b middle stem leaf, c upper stem leaf, d stem leaf in inflorescence area, e upper stem leaf in inflorescence area, f bract in inflorescence area, g T-shaped trichome, h simple one-celled hair, i stalked multicellular, glandular hair. Scale bars: a-d: 1mm, e, f: 0,5mm, g-i: 50 μ .

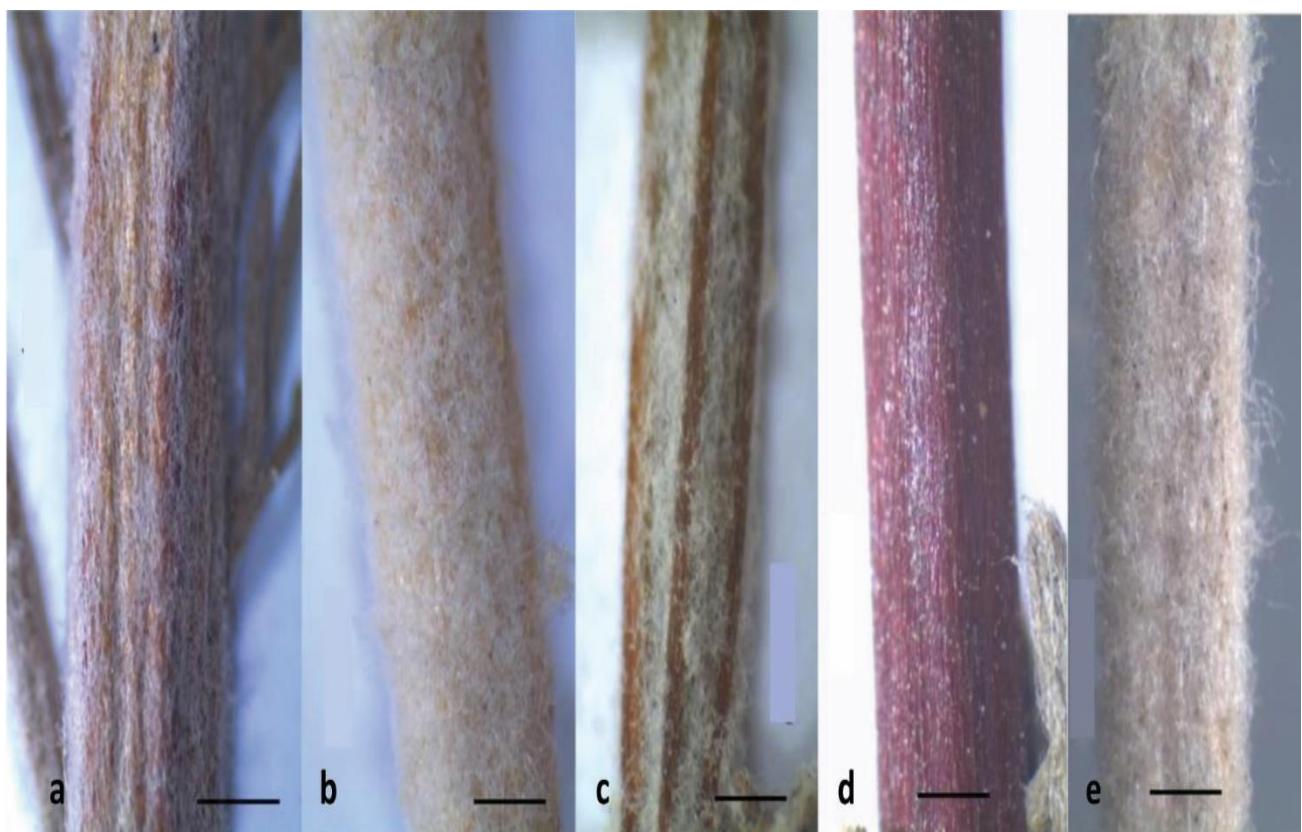


Fig. 8. Comparison of stems in the upper plant region. a *Artemisia quettensis* PODLECH, b *Artemisia sieberi* BESSER, c *Artemisia oliveriana* J. GAY EX BESSER, d *Artemisia turanica* KRASH., e *Artemisia santolina* SCHRENK. Scale bars a: 1mm, b-e: 0,5 mm.

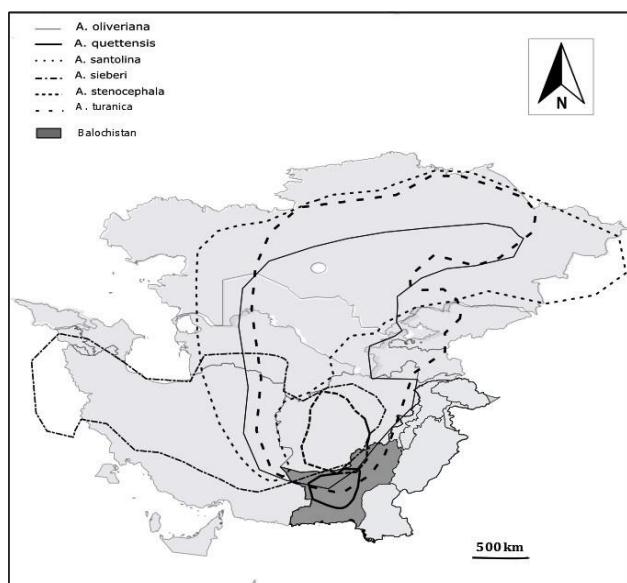


Fig. 9. Distribution map of the species *A. oliveriana*, *A. quettensis*, *A. santolina*, *A. sieberi* (incl. *A. dumosa*), *A. stenocephala*, *A. santolina*, *A. sieberi*, and *A. turanica* (incl. *A. eremophila*), designed by Dr J. P. Gruber (University of Salzburg).

Linking the distribution patterns of the species onto the precipitation values given by the niche modelling software package WorldClim (Tab. 1), *A. sieberi* is characterized by the lowest annual rainfall of calculated 73 mm and occupies the driest habitats at lower and middle altitudes. The appearance is distinct xeromorphic through early withered and deciduous leaves, branches, which are almost spiny at the end of growth, as well as the peeling bark. The calculated annual rainfall for *A. turanica* ranges from 137 mm to 228 mm, that for *A. quettensis* from 137 mm to 285 mm, and that for *A. oliveriana* and *A. stenocephala* from 252 mm to 265 mm. Whereas *A. turanica* grows more on dry, sandy and loess ground between 600m and 2,100m a.s.l., *A. oliveriana*, *A. stenocephala*, and *A. quettensis* prefer higher soil moisture and normally colonize higher altitudes from 900m to 2,400m a.s.l. The stem leaves in *A. turanica* are as early withered as in *A. sieberi*, however, in contrast to all other examined *Artemisia* species, the cross-section of leaflets is rounded. This reduction of the leaf surface can be interpreted as an adaption to drought. The stomata are slightly depressed, the lateral shoots are rather tenuous and flexuously. The stem leaves in *A. quettensis* and *A. oliveriana* are longer lasting and of considerable size; they have relatively large leaf areas. For *A. santolina*, an annual rainfall of 265 mm has been calculated. The perhaps new species for Pakistan has relatively mesomorphic appearance due to long and broadly decurrent laminas which are less lobed. All examined *Artemisia* species are more or less densely haired.

The populations of apparent hybrid origin are distributed over the surroundings of Quetta and cover an isohyet range from 137,6 mm to 265,6 mm. The annual precipitation does not seem to have an obvious impact on the divergence of putative hybrid populations. Nevertheless, we cannot exclude that all encountered *Artemisia* populations occupy also other, climatically different sites (which were not sampled). The climatic interrelationships are therefore not really conclusive and warrant further studies.

Discussion

The plant determination by morphometric methods resulted in six different *Artemisia* species and four putative hybrids. Most species are widespread and very common to central Asia, while some species, such as *A. stenocephala* and *A. quettensis*, are narrowly distributed, considered subendemic or endemic to Balochistan. As already mentioned, the taxonomic confirmation of *A. stenocephala* and *A. oliveriana* proved to be difficult both, on the basis of our collection, and through intensively consultation of the herbarium specimens from Iran and Afghanistan. We also found some specific characteristics of *A. quettensis* within those specimens, what is particularly remarkable taking into account the small area of distribution of *A. quettensis*.

The xeromorphic appearances of *A. sieberi* and *A. turanica* might be related to higher drought, however, for more detailed assessment, a smaller investigation network of more climate data and additional information on regional soil conditions, water supply, and grazing intensity would be necessary. High grazing pressure, for example, has similar effects as has drought (compare Grace, 1998; Diaz *et al.*, 2007; De Micco & Aronne, 2012). As mentioned above, *Artemisia* species are characterized by high phenotypic plasticity and high tendency of polyploidization and hybridization, both have widely been recognized to be important processes in species evolution (Grant, 1981; Arnold, 1997). Polyploidization was found as particularly active in *Seriphidia* (Podlech in Rechinger, 1986; Torrell & Vallès, 2001; Torrell *et al.*, 2003; Semple & Watanabe, 2009; Malik *et al.*, 2017), and polyploids often occupy a broader range of habitats, partly under extreme environmental conditions (Ehrendorfer, 1980; Rieseberg & Willis, 2007; Pellicer *et al.*, 2007, 2010, and references therein). The open landscape of the study area and changing habitat conditions, including a high number of disturbed niches, are generally good prerequisites for processes of polyploidization. Following Dolatary *et al.* (2013), a basic chromosome number of $x = 9$ apply to all *Seriphidia* from Iran, presenting different ploidy levels. For the hexaploid *A. quettensis*, a variable chromosome number (49+2, 3B, 51+6B, 54+3B/6x) was documented. It is also one of the rare species with simultaneous existence of B-chromosomes and aneuploidy (see also Watanabe, 2012). According to many authors (e.g. Stebbins, 1966; Wood *et al.*, 2009; Chehregani *et al.*, 2010; Pellicer *et al.*, 2010; De Stormea & Mason, 2014), the variation in chromosome number in a species can be considered incipient speciation. It can therefore be argued, the endemic *A. quettensis* is probably a young evolving species, the high polyploidy rate allowing colonization of new or a wider range of habitats in time (Stebbins, 1950; 1971; Grant, 1981; Beest *et al.*, 2012, and references therein). In this respect, Ahmad *et al.*, (2007) found prolonged seed dispersal and a persistent seed bank of *A. quettensis*, which may facilitate seed germination. Similar results were obtained by Gul *et al.*, (2007). However, overlapping characteristics with other *Artemisia* specimens from Iran and Afghanistan may indicate a wider distribution range of *A. quettensis*. Since we do not yet know the physiological limits of tolerance of this species and the other *Artemisia* species, we cannot yet assess their dispersal potential. In contrast to the endemic *A. quettensis*, the also hexaploid *A. santolina* (54/6x) is among the

widespread species within Central Asia and occurs in both, rather moist and very dry habitats. This may also be attributed to the greater ecological tolerance in polyploids (Soltis *et al.*, 2010, and references therein). Depending on the sampling site, *A. sieberi*, *A. oliveriana*, and *A. stenocephala* were reported both tetraploid and diploid (36/4x; 18/2x, see also Podlech & Bader, 1974 and Kursat *et al.*, 2015). Genetics, cell and stomata sizes, morphology, chemistry, and habitat preference may thereby modified (Valles & Torrell, 2001; Jalili *et al.*, 2013, and references therein). Whereas *A. stenocephala* and *A. oliveriana* also prefer mesic habitats, *A. sieberi* was found only on xeric sites. *A. turanica* is the only true diploid species (18, 36/2x, 18, 19/2x). The species prefers dry sites, and the stems are remarkable less densely tomentose. Unfortunately, we do not know the ploidy level of the specimens from Balochistan, which may differ from that in Iran. The determination of the ploidy level was not possible in our samples, as the genus *Artemisia* seems to be one of the few genera that are not suitable for flow analyses after desiccation. Actually, only background signals were obtained (J. Suda, 2009, pers. comm.).

However, whether morphological differences between ploidy levels resulting from polyploidization directly, phenotypic plasticity, or selection due to habitat differences is not always clear. Species also show different appearance in different phases of development. *Artemisia* plants have their full development and blooming period in general between September and November.

Podlech (in Rechinger, 1986) already pointed out to the role of hybridization in the evolution of *Artemisia* species, stimulated by their inherent propensity for polyploidization (Stebbins, 1985; McArthur *et al.*, 1988; Freeman *et al.*, 1991; Hegarty & Hiscock, 2005; Paun *et al.*, 2009). The high number of individuals per population, overlapping ancestors, wind transport of low weight pollen grains, same blooming period, and perennial life history might be favouring factors for polyploidization and hybridization in the study area. Although some samples of our collection are intermediate between putative parental species, the available morphological methods did not prove to be sufficient for unambiguously identifying true hybrids, further complicated by the absence of capitula, and considerable intraspecific variation (Haghghi *et al.*, 2014). We did not recognise continuous transitional forms between the two putative progenitors; moreover, the ploidy levels were partly different between the parental species (interpoloidy hybrids). This may be the case in *A. quettensis* x *A. oliveriana* and *A. quettensis* x *A. turanica*. Speciation and possible offspring of crossing of plants of different genotypes remain therefore problematic (Koutecký, 2008).

The limited budget and restrictive personal resources have proved to be a very serious obstacle. We hope, nevertheless, the present study will stimulate further efforts in floristic, cytogenetic, and molecular phylogenetic fields in order to get a better insight into the evolutionary diversification and speciation within the subgenus *Seriphidium*. However, it must also be kept in mind that the Asiatic *Seriphidia* are spread over a wide area, extending into countries with currently difficult political and economic situation, so that scientific fieldwork is often difficult and not save.

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