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Ophrys holoserica (Burm. f.) Greuter subsp. Shoufensis subsp. novo K. Addam & M. Bou-Hamdan (ORCHIDACEAE), A new world record from Lebanon

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Abstract
A new subspecies, *Ophrys holoserica* (Burm.f.) Greuter subsp. shoufensis subsp. novo K. Addam & M. Bou-Hamdan from the Orchidaceae family and related to the *Ophrys holoserica* group, is found and morphologically described as well as illustrated for the first time in Lebanon. The new subspecies share some main characteristics of *Ophrys holoserica* but contrasts in many taxonomic and morphological details such as the size of the flower (smaller), color of the labellum (blur yellow from up and very light brow from down), outlook (the whole flower is super fine) and habitat. The study discusses, examines, and offers taxonomic description, pertinent information, and photographs found all over 10 years of field work. It also includes observations, fresh collection, and one holotype.

Keywords: Bee orchid, *Ophrys holoserica* var shoufensis var. novo K. Addam & M. Bou-Hamdan, Orchidaceae, holocerica group, taxonomy, Middle East, Lebanon

1. Introduction
Lebanon is one of the most inspiring spots of conservation in the world [1]. It is an integral region in the Mediterranean Basin where it boasts one of the highest densities of floral diversity. One of the most incredible features about this country is the existence of such biodiversity in a very limited area. Lebanon occupies 0.007 % of the world's land surface area (10452 km2) and is a home to 1.11% of the world's recorded and catalogued plant species [2]. Its distinctive Mediterranean climate, geological breading and topographical diversity are the main factors that characterize it as a significant reserve for various sporadic, native and endemic species (particularly some of the very rare endemic orchids) [3].

The Orchidaceae, or the orchid family, is acknowledged as the largest of the monocotyledons and as the second largest family of flowering plants (angiosperms) after the family of composites (Asteraceae), with about 850 genera and some 25,000 species distributed nearly worldwide [3, 4]. Exhibiting incredible range of diversity in shape, size and color of their flowers, orchids are considered one of nature's most beautiful and gaudy appealing groups of flowering plants [5]. One of their hidden and significant usages is their medical procedure which is used in traditional medicine for treating several diseases such as circulatory, diabetes, cancer and others [6]. Even Dioscoridis and Aelius Galenus had revealed many of its medical uses (Unani medicine) like being a robust aphrodisiac [7].

Among this extravagant group of orchids is the genus Ophrys. "Ophrys" perhaps the name, 'oepoc' (eyebrow') in ancient Greek, was given to this genus of plants because of the intense lateral pilosity on their lips. Bauhin gave the name 'Ophirys' which was then modified after translation to 'Ophrys'. In classical botany the genus Ophrys is classified as follows: Family Orchidaceae -+ Subfamily Orchidoideae -+ Group -tribe Orchideae -+ Subgroup -Subtribe Orchidinae -+ Genus Ophrys various species [8, 9].

The genus Ophrys is commonly divided into two sections, Pseudophrys and Ophrys, on the basis of pollination type. Pseudophrys is characterized by abdominal pseudocopulation (pollinia attach to the extremity of the abdomen) and Ophrys performing cephalic pseudocopulation (pollinia attach to the head). The sections are mainly subdivided into species complexes and species groups based primarily on floral morphological characters (mainly the shape, color, hairiness and size of the labellum), which play a great role in the classification of the genus [8, 10]. Among the genus Ophrys, particularly the holoserica family, is our discovered new world record *Ophrys holoserica* subsp. shoufensis subsp. novo K. Addam & M. Bou-Hamdan whereby it is the core of our publication. Fig. 1, 2, 3.

Fig 1: The Whole Plant of *Ophrys holoserica* (Burm. f.) Greuter subsp. Shoufensis

Fig 2: The Flower's Full Face. of *Ophrys holoserica* (Burm. f.) Greuter subsp. shoufensis

The genus Ophrys ranges over a vast territory which extends from the Canary Islands in the west to Iran in the east, and from Scandinavia in the north to the Sahara in the south, with its main concentration in the Mediterranean region [11]. It is spread in Asia (Lebanon, Syria, Turkey and Palestine), also widely in Europe, (Spain, France Italy Greece and Cyprus) northward to Northern Ireland, eastward to Kavkaz Mt., and southward to North Africa (Algeria Tunisia Egypt Morocco). Ophrys species are distributed from Central to South Europe, North Africa, Asia Minor, up to the Caucasus Mountains, but mostly in the Mediterranean region [4].

In the floral realm, botanists show great interests in the orchid genus Ophrys as it is arguably the most thoroughly researched of all orchids [12] and remarkable for its pollinator mimicry through sexual deception which makes it a model system for evolutionary studies [13]. Furthermore, Ophrys is considered as the most significant and richest genus among the species of European and Mediterranean terrestrial orchids (ground orchids). This is so regarding its introgression, hybridization, and high genetic compatibility. In addition, this genus is noteworthy for its flower's distinctive and complex morphology. It is also notorious for its contentious taxonomy and problematic species delimitation [14, 9].

Because of the fascination of scientists for this charismatic genus and the introduction of new investigation methods and/or classification concepts, the number of described taxa has dramatically increased. Based on morphological characters and on molecular methods, this typical pervasive Ophrys is regarded as a monophyletic group which encompasses more than 250 taxa [10]. However, the taxonomic relationships in this genus are still ambiguous and controversial though many studies regarding morphological information, cytogenetic analysis, and molecular ITS nuclear DNA are offered [8, 2]. Because many of this species are considered to be of hybrid origin, and the floral morphology is highly variable, it is apparently problematic to distinguish its boundaries and construe its phylogenetic relationships [10, 15]. This problem is not only in this genus but also found in other genera of orchid species.

For the last twenty years, orchids have grabbed the attention of botanists, particularly orchidologists. As a result, new articles as well as books comprising a multitude of new orchids' names have been published. This leads to a description of new taxa (species, subspecies, varieties and forms) that confused these orchidologists. A correct description of these novel taxa is attempted, but others are still debatable whereby they are specified with a particular rank or sub-rank which may vanish by time. Morphological characteristics such as the size and color of the flower are the main factors in a correct classification of the orchids at their natural habitat. Other flower's characteristics and flowering time are determined only at the spot.

In the early years, authors clearly describe orchids as subspecies while in the last twenty years, these new taxa are described as completely specific ranks or species. A taxonomical concept is regarded whereby the species described first is considered as the nominate species and hence classified as subspecies or lowered to specific rank. The advantage of this classification is that closely related species can be easily and reliably followed by most orchid specialists nowadays. Till now, no one is winning the debate concerning the taxonomy, nomenclature, and classification of the new taxa of Ophrys.

With the abundance of nomenclatures and classifications, we selected two classifications whereby our adoption was built on one of them in order to determine the nomenclature of our new holoserica. One of these classifications goes back to Delforge and Paulus which displays a more or less simple system comprising only species and varieties which are based on biological facts. The other classification goes back to Karel C.A.J. Kreutz which resembles a more complex system made up of subspecies. A very difficult decision was to choose and adopt a classification to name our new holoserica. Finally, after a lot of readings and comparisons amid the classifications of the holoserica's nomenclatures, we (K. Addam & M. Bou-Hamdan), decided to follow that of the Karel C.A.J. Kreutz. This classification has been modified in many cases for the sake of practical clarity and identification of species for scientists and orchidologists that produce hardly tangible variances [16].

Lebanon is considered as a reserve for many unique and endemic native orchids that includes a well-known global center of plant diversity. Geological upbringing, favorable climate, and topographical diversity of Lebanon contribute greatly to the richness of its orchids [4]. It consists of two mountain ranges: The Mount Lebanon and Anti-Lebanon Chains with 73% of the total mountainous area where most orchids grow [17].

Mount Lebanon is one of the regional hotspots [18, 19] whereby 85% of orchids are found during our 21 years of field work. Few researchers worked specifically in the domain of the Lebanese Orchidaceae family such as K. Addam and M. Bou-Hamdan [15, 11, 4, 1, 6, 18, 19, 9, 20], Haber R, M. & Haber M.S., Kreutz and others. Haber & Haber [21, 16] wrote the only book that illustrated specifically the Lebanese orchids entitled "Orchids of Lebanon" [21]. Many novelties were suggested for Lebanon, but without any further discussions between the parties. After spending many years searching for publications that might contain these new pictured species (mentioned in the book), we concluded that no publications were found or mentioned even in the book's bibliography for these authors regarding the new species even no specimens were discernible to exist for them in any herbarium.

Novelties mentioned in their book from the holoserica family were *Ophrys holoserica* subsp. helios, aramaeorum, heterochila, gresivaudanica and episcopalis [21]. Some

novelties found in the list written by Kreutz about the Lebanese orchids transpired to be a personal field inventory rather than an exhaustive compilation based on historical bibliography. A checklist for Lebanon and Syria was done by Kreutz (2006), but it is limited to the taxa encountered by him and is now outdated.

In 2015, Vela, E. and Viglione wrote a proposal of a national checklist for the Lebanese Orchidaceae family. Indeed, this checklist needs to be updated because it does not contain a lot of new discovered and published species by K. Addam and M. Bou Hamdan before the proposal of Vela, E. and Viglione as well as new world endemic records which we have recently validated after their proposal. These new discoveries were published after four years in 2015, 2016, 2017 & 2018. Nonetheless, we disagree with the classification of this checklist because it momentously conflicts with our nomenclature and classification interest [19].

All the other nomenclatural novelties are also considered, especially the important work of Baumann and Baumann, where he announced the discovery of *Ophrys holoserica* subsp. libanotica B.Baumann & H.Baumann [22].

Other scientists worked on the Lebanese flora in general [23, 24, 25] and spoken of the Lebanese Orchidaceae family among their other findings of the Lebanese flora. It is obvious that the nomenclatures of some species as well as the classifications of other Orchids' families are not accepted anymore [26] in some old Lebanese flora books [23, 25]. These books contain some outdated information whereby the existence of other new members and varieties related to this species are not mentioned. Tohme and Tohme's book [21] has palpable mistakes not only in the flora but also in identifying the names of the Orchid species.

These scientists mentioned some species that are considered now as new ones but recognized them as one species. Since then, a lot of discoveries were made in the field of Orchidology with the advancement in technology whether in the fields of genetics or pollination. For instance, some scientists mentioned that we have only Ophrys fuciflora in Lebanon, but according to the new classifications, we already have the Ophrys bornmuelleri group [16], *Ophrys holoserica* group, Ophrys tetraloniae group, Ophrys fuciflora group, Ophrys heldreichii group with respect to Delforge classification and others. According to Delforge, bornmuelleri group varies in the eastern Mediterranean and its members participate in an idiosyncratic group. However, the small dissimilarities of these plants compared to the central European Ophrys do not substantiate separation into another sub-group [27]. Others like Kreutz gave a more complex situation for his new enlarged long classification of the holoserica group turning it into subspecies, and so that scientists and people can see it clearer and avoid confusion in identification [16].

Regardless all these groups, species and subspecies that have red sepals were assumed as Ophrys fuciflora which make the information found in these books wrong, but even though they are still widely accepted and considered as very significant references for many other species still can be trusted many publications. Their importance lies in providing perspectives on the species, classifications, generic concepts and many extinct and still valid nomenclatures currently employed in orchid systematics of the Lebanese flora [1, 18].

2. Materials and Methods

Ophrys holoserica subsp. shoufensis subsp. novo K. Addam & M. Bou-Hamdan was found in two places in Gharifeh (Mount Lebanon). It was discovered and pictured for the first time at 5/V/2008 but was not identified then. After six years at 26/IV/2014, it was found in another place in the same village. This species of ophrys is very rare (in Lebanon) and very difficult to discover, but it is straightforwardly distinguished and detected during the field work search due to its unblemished dissimilarity in the color of very fine labellium and its early blooming.

3. Results

In this study, *Ophrys holoserica* (Burm.f.) Greuter subsp. shoufensis subsp. novo K. Addam & M. Bou-Hamdan is discovered, identified, and added to the Lebanese flora for the first time. It resembles *Ophrys holoserica* but differs in some taxonomic, morphologic and phenology attributes.

Fig 3: The Flower's Profile and Full Face of *Ophrys holoserica* (Burm.f.) Greuter subsp. shoufensis

3.1 Description of the *Ophrys holoserica* group

Ophrys holoserica is an orchid species identified by (Burm.f.) Greuter in 1967 [28]. The group of this ophrys contains about 52 subspecies [29]. It is one of the most populous comprising members related to Ophrys fuciflora, also known as Ophrys holoserica, displaying a specifically broad distribution in central Europe.

Holoserica group has attractive and complex flowers characterized by the strong red-violet perigon (perianth), the square lip with flat edges and the large, and upwardly turned three-toothed appendix. The petals are thinly pilose and usually small and at a distance from each other. Morphologically, the holoserica group is very closely related to the oestrifera and heldreichii groups, slightly varying in the borders of the lips, which in holoserica are flattened outwards and in the latter turned backwards, giving a cylindrical 'scolopacidae'. Furthermore, within the same population, Ophrys heterochila belonging to the holoserica group and Ophrys calypsus belonging to the heldreichii group, demonstrate forms with flowers that are sometimes cylindrical and amphora-shaped, and sometimes trapezoidal [30].

3.2 Description of *Ophrys holoserica* subsp. shoufensis subsp. novo K. Addam & M. Bou-Hamdan.

(All the measures are done on the holotype)

The new *Ophrys holoserica* (Burm.f.) Greuter subsp. shoufensis subsp. novo K. Addam & M. Bou-Hamdan is 39

cm tall. Its bract is 22.9x8.5mm longer than its flower. Its leaves has 4 basal 124 x 20.4 mm and 2 smaller stems. The inflorescence is not dense. It has 4 flowers (medium-sized for the group); dorsal sepal 11.78 x 5.32mm; lateral sepal 12.30 x 5.65 mm; dark purplish and light rose (sometimes washed white at tip and purple at the base), green line in the middle, oval spreading concave and slightly turned back petal of 3.69 x 3.17xmm. They are small, villous, erect, triangular rounded, separated, color as sepals or slightly darker (sometimes becomes lighter at the tip). The lip is pendent, (near) horizontal, entire 14.69 x12.19 mm long with appendix, 14.11mm without, ± convex, appearing trapezoid or square. It is velvety, light yellowish brown to slightly dark brown, with a complete sub marginal band of dense, rather long, white yellowish hairs, with one small, conical basal swelling, ± pointed, erect, often divergent and curved, hairless inside with yellowish green color from up and light yellowish brown from down. The margin of the lip is hairless, dark yellow (light yellowish brown while moving up), sometimes scalloped, spreading and then reflexed, ± strongly on distal half. The speculum is 6.02x7.2 mm, basal, extremely very light yellowish brown, blur and very difficult to be distinguished as it moves down towards the median lobes separated from the basal field by a blur black line, in the form of trapezoid or square surrounding the basal field; basal field 3.55 x 4.76 mm, orange color at the board with the speculum then become light brown when moving up, often extended by lateral branches, by 2 ocelli, appendage 4.05mm long, broad, pointed, with 3 teeth, pure yellow, angled upwards, inserted into a deep notch. It has a stigmatic cavity of small, transverse, 3.8 x 3.5mm high, sometimes slightly separated from lip, bordered by 2 rounded to elliptical, shiny, greenish yellow pseudo-eyes (extremely difficult to be recognized); ovarium 23.48 x2.76mm, staminodial points often present; pollinia 4.42mm, pollinium 1.6x0.94mm, column rather small, ± acuminate, forming an open angle with lip. Fig. 1, 2, 3.

Fig 4: *Ophrys holoserica* subsp. shoufensis; flower parts; flower viewed from front and back; floral organs; dorsal and lateral sepals; petals; bract; ovary; pollinia; pollinium; labellium and speculum.

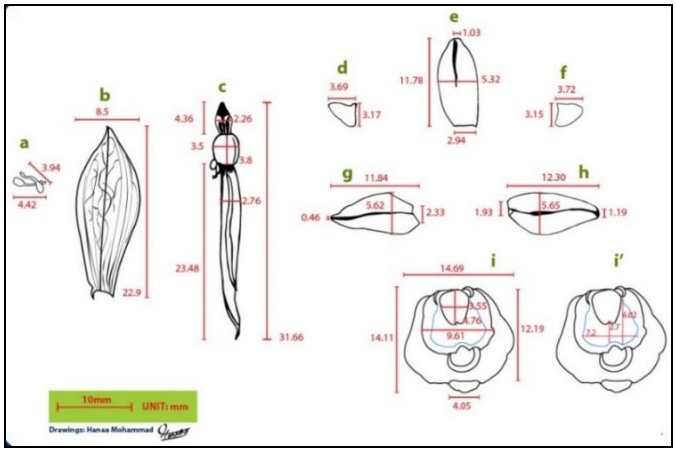

Fig 5: *Ophrys holoserica* subsp. shoufensis subsp. flower organs: flower viewed from above; (e) dorsal and (h,g) lateral sepals; (d) petals; (b) bract; (c) ovary; (c) pollinia; (c) pollinium;(i) labellium and (i') speculum (from Addam & Bou-Hamdan S.N. 26-4-14-78-001 (Holotype AUL, Lebanon, K. Addam Herbarium); Drawn By: Hanaa Mohammad).

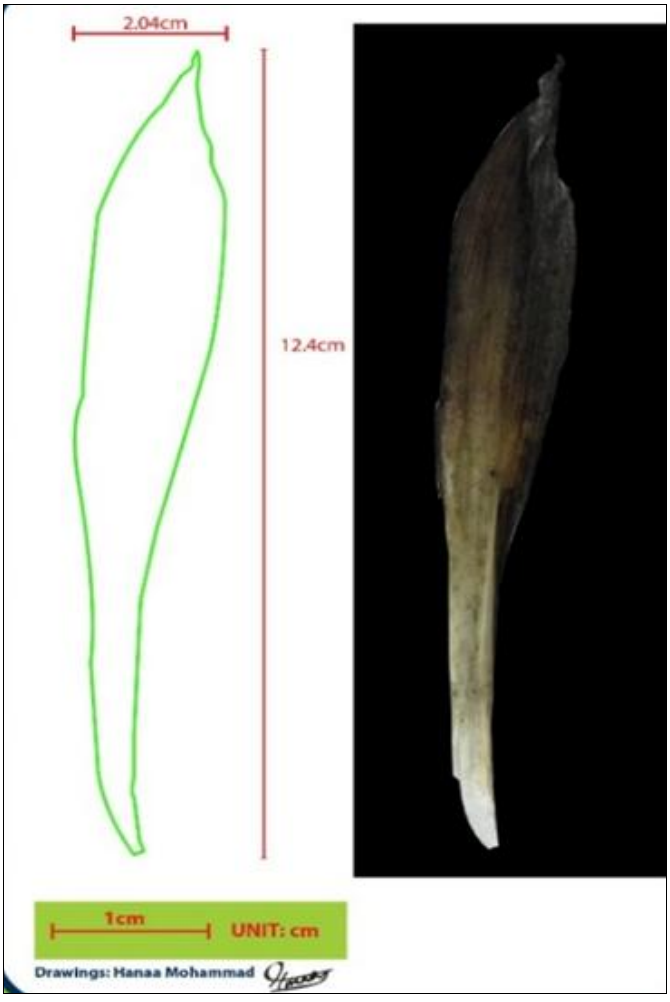

Fig 6: Leaf and Whole Plant of *Ophrys holoserica* subsp. shoufensis

Fig 7: Taxonomic Dissection for the petal, sepal and bract

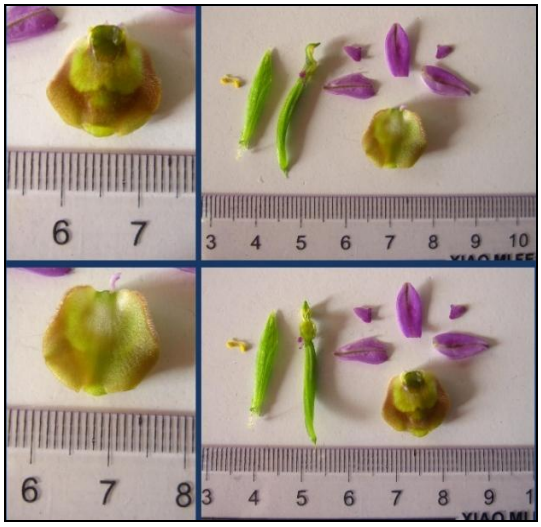

Fig 8: Taxonomic Dissection for all Parts of *Ophrys holoserica* subsp. Shoufensis

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Fig 9: Non-blooming flowers, labellium, and ovarium

3.3 Flowering Season

Late IV -V. Always earlier than other members of the holoserica groups.

3.4 Location

Mount-Lebanon. Gharifeh (Kaza of Chouf in mohafaza of Mount-Lebanon) 56 km away from Beirut. Gharifeh, N 33° 38’ 78” EO 35° 32’ 204” Alt 666m “Al Shaareen”. Gharifeh N 33° 37’ 208” EO 35° 33’ 31” Alt 820m “Abou Soultan Valley”. Fig. 10

Fig 10: Distribution of *Ophrys holoserica* subsp. shoufensis in Lebanon

3.5 Habitat

Ophrys holoserica subsp. shoufensis subsp. novo K. Addam & M Bou-Hamdan is a perennial growing from the end May to April. The species is hermaphrodite (has both male and female organs) and is pollinated by Insects. Suitable for: light (sandy), medium (loamy) and heavy (clay) soils. Suitable pH: acid, neutral and basic (alkaline) soils. It can grow in semi-shade (light woodland) or no shade. It prefers fresh dry calcareous basic-rich soils, poor meadows, open rocky place and woodlands, under pine trees and direct sunlight. It is noteworthy that it does not grow and bloom every year which makes it very challenging to be found if there are no adjusted signs. Moreover, it is discovered always to grow between Sarcopoterium spinosum and it is occasionally and almost covered by them.

3.6 Etymology
The subspecies epithet is attributed to Gharifeh village. There are many legends about the origin of the name of Gharifeh but many agree that it comes from Syria and means "cliff" or "extended mountain".

3.7 Voucher Specimen
A dried sample voucher specimen of *Ophrys holoserica* subsp. shoufensis subsp. novo K. Addam & M. Bou-Hamdan was deposited in K. Addam's Herbarium Arts, Sciences and Technology University in Lebanon, serial number (26-4-14-78-001), collected by Dr. Addam at 26/IV/2014. Fig 11

Fig 11: Dried Specimen (Holotype) of *Ophrys holoserica* (Burm.f.) Greuter subsp. shoufensis subsp. novo Deposited at K. Addam Herbarium, AUL Lebanon SN (26-4-14-78-001)

3.8 Pictures and Drawings
The pictures were captured by Dr. K. Addam and M. Bou-Hamdan and the drawings were drawn by Hanaa Mohammad.

4. Status and Threat
A multitude of factors are threatening the orchids. This exceptional flora is damaged and ruined by tourists, urban expansion and proliferation of summer resorts in the mountains, overcollection of the aromatic and medicinal herbs, overgrazing, and forest fires. Consequently, many orchid species are regarded to be extinct whether directly or indirectly of human activities that are acknowledged as the chief menace to biodiversity. Countless other reasons are recorded worldwide and can be related to Lebanon such as inappropriate fire regimes like planned wildfires to get charcoal, barbecue fires, home fires or chimney fires or garbage fires which lead to burning hundreds of orchids yearly. In addition to deforestation that results from gathering of firewood by the local people and the selective logging of timber in forests often affect the survival of the epiphytic orchids [1, 31].

Ophrys holoserica subsp. shoufensis subsp. novo is a very sporadic orchid that requires a stern protection because it is found and grown near houses in villages that contain very fast urbanization.

5. Discussion
The new subspecies is found for the first time in 5- V- 2008 and for the second time in 26- IV- 2014. The new subspecies appears to grow in light (sandy), medium (loamy) and heavy (clay) soils, semi-shade (light woodland) or no shade (prefers fresh dry calcareous basic-rich soils), poor meadows, open rocky place and woodlands, under pine trees and direct sunlight. It is noteworthy that it does not grow and bloom every year which makes it very difficult to be found if adjusted signs are lacked. In addition, it is discovered that it always grows between Sarcopoterium spinosum bushes and is sometimes covered almost by them. It blooms almost at the end of April till the end of May which is earlier than other members of the holoserica groups.

Each population of *Ophrys holoserica* subsp. shoufensis subsp. novo K. Addam & M. Bou-Hamdan was normally observed to share its habitat with *Sarcopterium* spinosum, Potrium verrucosum, *Pinus brutia*, *Pistacia palaestina*, *Daucus carota*, Arum palaestina, *Atractylis comosa*, *Bellis sylvestris*, *Carthamus tenuis*, Cichorium intybus, *Echinops adenocaulos*, *Helichrysum sanguineum*, *Pallenis spinosa*, *Notobasis syriaca*, Tragopogon longirostris, *Anchusa azurea*. Stellaria media, Cistus criticus, Pisum fulvum, Psoralea *bituminous*, Trigonella berythea, *Vicia angustifolia*, Dorycnium hirsutum, *Calicotome villosa*, *Centaurium erythraea*, Gladiolus segetum, *Teucrium polium*, Teucrium stachyophyllum, Allium trifoliatum, Orchis coriophora fragrans, Orchis sancta, *Spiranthes autumnalis*, Orchis tridentate, *Serapias vomeracea*, Clematis *flamulla*, *Rhamnus* alaternus, Pirus syriaca, Osyris alba, *Thesium humile*, *Bellardia trixago* and many others.

The general structure of *Ophrys holoserica* (Burm.f.) Greuter subsp. shoufensis subsp. novo K. Addam & M. Bou-Hamdan is similar to the plant structures of the holoserica group. It is characterised by its lip. The lip is pendent, (near) horizontal, appearing trapezoid or square, velvety, light yellowish brown to slightly dark brown Fig. 3, with a complete sub marginal band of dense, rather long, white yellowish hairs, with one small, conical basal swelling, ± pointed, erect, often divergent and curved, hairless inside with yellowish green color from up and light yellowish brown from down ; margin of lip hairless, dark yellow (light yellowish brown while moving up), sometimes scalloped, spreading and then reflexed, ± strongly on distal half; speculum, basal, extremely very light yellowish brown, blur and very difficult to be distinguished as it moves down towards the median lobes separated from the basal field by a blur black line, in the form of trapezoid or square surrounding the basal field; basal field, orange color at the board with the speculum then become light brown when moving up. Fig. 2, 3.

6. Recommendations

Ophrys holoserica (Burm.f.) Greuter subsp. shoufensis subsp. novo K. Addam & M. Bou-Hamdan is a very rare and endangered species. Many problems have shown to be essential factors in endangering the existence of this species. We recommend some solutions for these existing problems that need to be stopped and eliminated but before that we need to determine the threatening factors as well as the preventions against them [17, 31]. These reasons have expansively affected flora especially orchids all over Lebanon. They include intensive grazing pressure from mammalian herbivores such as sheep, goats and cattle in addition to fast urbanization which leads to the clearance of large areas of bushlands and forests that host these species. Not to mention inappropriate fire regimes such as wildfires and barbecue fires. Recommendations that should be undertaken against these factors are the implementation of awareness campaigns that introduce educational and social events whereby news jacking and distributing brochures are provided for people in general and village men/women in particular.

7. Conclusion

Ophrys holoserica (Burm.f.) Greuter subsp. shoufensis subsp. novo K. Addam & M. Bou-Hamdan joined the Lebanese flora and specifically the world Holoserica group. After 10 years of fieldwork observing the aforementioned ophrys, its existence was proved by its multitude of locations Fig.10, illustrated morphological description and phenology. One voucher specimen (holotype) for the new subspecies (represented dried sample) of the plant were deposited in K. Addam's Herbarium Arts, Sciences & Technology in Lebanon collected by Dr. K. Addam and identified by K. Addam & M. Bou-Hamdan & K. Addam. Fig. 11

8. Acknowledgement

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