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Description and population change of *Schistonchus caprifici*, the nematode associated with the pollinator fig wasp, *Blastophaga psenes*, in Estahban, Iran

Maryam KOLAEI, Mohammad ABDOLLAHI, Alireza MONFARED

Abstract

In order to identify and description of the nematode associated with pollinator fig wasp (*Blastophaga psenes*) in Estahban, Fars Province, Iran, the syconia of caprifig (*Ficus carica* var. *sylvestris*) were collected from Bare-Paeen region, during 2012-2013. The nematodes were extracted from samples using incubation technique. The chopped figs were placed in petri plates containing sterile water then incubated at dark for 48 hrs. Extracted nematodes were fixed and transferred to glycerin and the permanent slides were prepared. The fixed nematodes were examined by light microscope, equipped with drawing tube and were identified, considering the morphologic and morphometric characters, with the help of scientific keys and references. In this study, *Schistonchus caprifici* was isolated from studied syconia. The isolated nematode is describing for the first time in Iran. To determining the population density as well as the sex ratio, numbers of extracted female and male nematodes were counted separately. Maximum population density per syconium recorded as 751 and 515.5 individual for female and male nematodes, respectively. The female to male nematodes sex ratio was about 3:2.

Key words: *Blastophaga psenes*, Caprifig, Nematode, Pollinator fig wasp, *Schistonchus caprifici*.

Zusammenfassung

Zur Identifizierung und Beschreibung der Nematode, die mit dem Bestäuber, der Feigenwespe (*Blastophaga psenes*) assoziiert ist, wurden in Estahban, der Provinz Fars, im Iran, die Syconia der Kaprifikation (*Ficus carica* var. *sylvestris*) in der Bare-Paen-Region während 2012-2013 gesammelt.

Die Nematoden wurden aus den Proben unter Verwendung der Inkubations-Technik extrahiert. Die gehackten Feigen wurden in Petrischalen, die steriles Wasser enthielten, bei Dunkelheit für 48 Stunden inkubiert. Die extrahierten Nematoden wurden fixiert und in Glycerin übertragen und Permanentträger wurden angefertigt. Die fixierten Nematoden wurden im Lichtmikroskop mit Ausstattung eines Zeichen-Tubus, unter Berücksichtigung der morphologischen und morphometrische Merkmale, und der Verwendung wissenschaftlicher Schlüssel und Referenzen untersucht. In dieser Studie wurde *Schistonchus caprifici* bei der Untersuchung der Syconia nicht berücksichtigt. Die isolierte Nematode wird zum ersten Mal aus dem Iran beschrieben. Zur Bestimmung der Populationsdichte sowie des Geschlechterverhältnisses, wurden die Anzahl von extrahierten weiblichen und männlichen Nematoden separat gezählt, sowie die maximale Populationsdichte pro Syconium aufgezeichnet, 751 weibliche und 515,5 männliche Nematoden wurden festgestellt, was ein Geschlechtsverhältnis von ungefähr 3:2 ergibt.

Introduction

Turkey, Egypt, Algeria, Morocco and Iran, are occupied the first to fifth positions of fig cultivation in the world, respectively (FAO 2012). Fars province is one of the most important regions of fig cultivation, and Estahban is the most important region of dried fig producer and exporter in Fars province (JAVANMARD 2010). Smyrna fig group is the most available varieties of edible figs, *Ficus carica* var. *smyrna*, in Iran, which need pollination for fertilization and fruiting (FAGHIH & SABETSARVESTANI 2001). The process of pollen transferring from the stamens of caprifig fruit to the surface of stigma of long style female flowers, which is so-called caprifigation, is carried out by the pollinator wasp (SHOJAEIAN 1994). In Estahban, two types of wasps are symbiont with the caprifig fruits. One is the black wasp, *Blastophaga psenes* (L.) (Hymenoptera, Agaonidae) with a short ovipositor and the other is the yellow wasp, *Philotrypesis caricae* (L.) (Hymenoptera, Torymidae) with a long ovipositor. Despite gardeners' perception, not only *P. caricae* does not have any role in pollination, but it is an egg parasite of *B. psenes* (KHODAPARAST & MONFARED 2010).

Some of nematodes are related to some invertebrates such as insects, ticks, mites and snails. The relationship between nematodes and invertebrates may be variable from the random relationship in the form of phoretic or commensal, to obligate parasite and pathogen (ABDOLLAHI 2013). Among nematodes, some members of the families

Rhabditidae, Diplogasteridae, Cephalobidae, Aphelenchoididae, Monochidae, Plectidae, Strongylidae, and Tylenchidae, have a phoretic relationship with insects (RINKER & BLOOM 1982).

Most of Aphelenchoididae, have two types of phoretic and facultative relationships with insects and are related to Scolytidae bark beetle, Cerambycidae long-horn beetle, and weevil beetle of the family Curculionidae. The genera *Aphelenchoides*, *Bursaphelenchus*, and *Schistonchus* have a phoretic relationship with insects (GREWAL et al. 2006). In family Diplogasteridae, the species *Parasitodiplogaster sycophilus* has been reported as a parasite of the females of *Elisabethiella stuckenbergi*, the pollinator wasp, as well as the fruits of fig, *Ficus thonningii*, in Zimbabwe (POINAR 1979). So far, twenty one species of *Schistonchus* have been isolated from the genus *Ficus*, (COBB et al. 1927, KUMARI & REDDY 1984, REDDY & RAO 1984, KHAIRI & GOLDANSAZ 1998, VOVLAS et al. 1988, DECRAPPEO & GIBLIN-DAVIS 2001, ANAND 2002, ZENG et al. 2007, 2010, 2011, 2013a, 2013b, 2013c, DAVIES et al. 2010, 2013, BARTHOLOMAEUS et al. 2012). So far, *S. caprifici*, has been reported from Algeria, Turkey, Italy, India and Iran. In Iran, this nematode has been isolated from the hemocoel of the female fig wasp, *B. psenes*, and fig fruit as well (KHAIRI & GOLDANSAZ 1998).

The biology of *S. caprifici* has been studied by VOVLAS et al. (1992). According to their study, the female *B. psenes* enters into syconia holes of fig fruits and pollinates the female florets. At the time of oviposition, *S. caprifici* is deposited near the florets. Then nematode penetrates and feeds on the parenchymal tissue of the floret and reproduce. They can also migrate out of the florets. Eggs of nematode hatched in hemocoel of female wasp and newly emerged larva feed from floret tissue. Nematode larva also infect the hemocoel of wasp larvae, and the life cycle of the both nematode and wasp will complete simultaneously. Then, the male wasp which has not infected with nematode, mates with the female, and the fertilized female wasp which carries the nematode in her hemocoel, leaves the syconium and in this way the life cycle is completed. All life stages of this nematode including egg, larva, male and female, have been found in both spring and summer syconia (VOVLAS et al. 1992). Based on KHAIRI & GOLDANSAZ (1998), during the oviposition of mature wasp, the females *S. caprifici* are transmitted from infected inflorescence to healthy inflorescences and will develop and reproduce in *Blastophaga* hemocoel. DECRAPPEO & GIBLIN-DAVIS (2001) have recovered *S. aureus* associated with *P. mexicanus* and syconia of *F. aurea* and *S. laevigatus* associated with *Pegoscapus* sp. and *Ficus laevigata*.

GIBLIN-DAVIS et al. (1995), have studied the nematodes associated with the pollinator fig wasp, *Pegoscapus assuetus* (Hymenoptera, Agaonidae) and syconia of native Floridan fig, *Ficus laevigata*. According to their study, the J3s of *Parasitodiplogaster* sp. are transported by mated female wasp into the cavity of a phase B syconium, then they develop to J4 and their size increase in the hemocoel of female wasp. Subsequently, the female begins to pollinate and oviposit in female florets. Then the J4s emerge from the wasp and develop to adults in phase B or early phase C syconium, that mate and lay eggs. Eggs, J2s and J3s are produce in the cavity of syconia in the mid phase C. All stages except the J3s have a large stoma armed with two prominent tooth that help the nematode to feed. At late phase C and phase D, the nematodes are in J3 stage with an

average number of 74 and 59, respectively. As the next generation of wasps emerge from their galls in phase D syconia, they may be infected by new J3s of nematode. None of any stages of this nematode have been observed in floret tissues, but almost all of J3s were observed in syconium cavity. In their study, they have also observed that mated females of another nematode, *Schistonchus* sp., infect the female wasps which is appeared around the cavity of syconium phase B. Female nematode merges from the body of wasp and parasitize the immature male florets resulting the abnormality of the anther filament and pollen. There is at least one generation of this nematode in the male florets. At the late phase C syconia, the entomogenous females appear consequent to maturation of fig wasps in their galls. Because the description of this nematode has not been done in Iran, this study was conducted to identify and describe the nematode associated with the pollinator fig wasp in fig gardens of Estahban, Iran.

Materials and Methods

During the frequent visits of caprifig gardens of Estahban in 2012 and 2013, twenty syconia of winter, spring and summer (fall) caprifigs were collected at the sampling periods of early spring, late spring and late summer, respectively (Tab 1).

Tab 1. Sampling times of caprifigs in Estahban.

Season	Sampling date	Caprifig growth season
Spring	April 6, 2013	Winter caprifig
Spring	April 12, 2013	Winter caprifig
Spring	April 19, 2013	Winter caprifig
Spring	June 6, 2013	Spring caprifig
Spring	June 12, 2013	Spring caprifig
Spring	June 20, 2013	Spring caprifig
Summer	September 3, 2013	Summer or autumn caprifig
Summer	September 12, 2013	Summer or autumn caprifig
Summer	September 19, 2013	Summer or autumn caprifig

Samples were transferred to the laboratory and kept in the refrigerator until extraction. To extract the nematode, after surface sterilization, caprifigs chopped using a scalpel, and then were suspended in sterile distilled water and placed in dark at 25°C. After 48 hours, the water passed through a 60 mesh sieve which nested over a 500 mesh sieve. The collected nematodes were killed by heat and adding 4% formaldehyde solution, and

mounted in glycerin using the Seinhorst method (SEINHORST 1959, 1962, 1966). Photomicrographs were taken with a USB AM7023 Dino-Eye digital camera coupled to an Olympus BX31 biological light microscope and drawings were made with the aid of a drawing tube attached to the same microscope. Measurements were made using Dino-Lite Pro software. Nematode counting carried out by using an Olympus SZX10 stereo microscope.

Results and discussion

S. caprifici was isolated from syconia of all the winter, spring and summer (fall) generations of caprfig in Estahban. Identification of the species was done using the key of *Schistonchus* species that has been provided by VOVLAS & LARIZZA (1996). Present study is the first description of the species from Iran. Specifications of identified species are as follows:

Description: Measurements in comparison to population described by VOVLAS & LARIZZA (1996) is given in Tab 2.

Tab 2: Morphometric characteristics of Estahban population of *Schistonchus caprifici*.

Characters	Estahban region		VOVLAS & LARIZZA (1996)
Sex	Female	Male	Female
n	10	5	10
L	445.5 ± 25.8 (412- 493)	351 ± 15 (340- 368)	476.3 ± 31 (421-500)
a	31.7 ± 2.8 (28.8- 36.2)	23.4 ± 2.5 (21.8 ± 25.7)	35.4 ± 3.1 (29.4-40.2)
b	5.3 ± 0.3 (4.9- 5.8)	5.5 ± 0.3 (5.2- 5.9)	6.4 ± 0.6 (5.7-7.1)
b'	3.3 ± 0.4 (2.8- 4.1)	3.3 ± 0.4 (3.1- 3.6)	4.2 ± 0.4 (3.7-5)
c	13.6 ± 0.9 (11.5- 14.5)	11.9 ± 0.7 (11.3- 12.3)	12.6 ± 1.3 (10.5-14.8)
c'	3.6 ± 0.3 (3.2- 4.1)	1.8 ± 0.5 (1.6- 1.9)	-
V	72.1 ± 1.8 (68.4- 75.7)	-	69.6 ± 1.4 (69.7-71.9)
St	21 ± 0.6 (20- 22)	21 ± 1 (20- 21.9)	22.8 ± 0.5 (22.1-23.4)
Conus	12.6 ± 0.8 (11.1- 13.3)	12.2 ± 0.7 (11.5- 12.9)	-
M	59.7 ± 4.3 (50.5- 63.8)	58.3 ± 3.4 (57.5- 58.9)	-
O	84.2 ± 5.5 (75- 96)	63.7 ± 5.1 (58- 68)	-
Oesophageal glands end	137.8 ± 14.3 (113- 155)	106.3 ± 2.9 (103- 108)	-
Oesophageal glands overlap	53.4 ± 14.1 (32- 70)	42.6 ± 22.4 (35- 50)	-
Median bulb	52.3 ± 2.5 (48- 56)	41.3 ± 1.2 (40- 42)	49.8 ± 1.0 (48.1-51.3)
MB	62.3 ± 3.9 (56.8- 68.7)	65.1 ± 3.7 (61.8- 69)	-

Characters	Estahban region		VOVLAS & LARIZZA (1996)
Median bulb width	8.8 ± 0.7 (7.6- 9.8)	9 ± 0.5 (8.5- 9.3)	-
Excretory pore	74.8 ± 5.9 (66- 84)	63 ± 4.2 (60- 66)	-
Nerve ring	68.1 ± 6.7 (58- 79)	54.7 ± 4.2 (50- 58)	-
Head-vulva	320.9 ± 17.8 (301- 356)	-	-
Head-anus	412.5 ± 22.9 (380.4- 450)	121 ± 16.7 (310- 338)	-
Vulva-anus	91.6 ± 10.9 (68.4- 107.8)	-	-
Tail length	33.1 ± 3.8 (30- 43)	29.5 ± 9.0 (28.5- 30)	38.1 ± 5 (31.8-44.8)
Tail/Vulva-anus	0.4 ± 0.1 (0.3- 0.5)	-	-
Body width	14.1 ± 1.3 (11.4- 16.5)	15 ± 0.8 (14.3- 15.8)	135 ± 0.8 (12.3-14.3)
Vulval body width	13.9 ± 1.0 (12.3- 16)	-	-
Anal body width	9.3 ± 0.7 (8.5- 10.6)	16.1 ± 1.2 (15.4- 17.5)	-
P.V.U.S	9.8 ± 1.9 (6.4- 12.7)	-	-
P.V.U.S/VBW	1.4 ± 2.3 (0.40- 8.1)	-	-
Vagina	9.0 ± 0.8 (7.9- 10)	-	-
Vagina/VBW	0.7 ± 0.1 (0.6- 0.7)	-	-
Lateral field width	3/3 ± 0.5 (2.6- 3.8)	3.2 ± 0.4 (2.8- 3.6)	-
Lateral field/BW	23.9 ± 2.3 (19.5- 26)	21.6 ± 2.1 (18.7- 22.8)	-
Lip region width	5.3 ± 0.3 (5- 6.1)	5.7 ± 0.4 (5.4- 6.2)	-
Lip region height	2.4 ± 0.1 (2.2- 2.7)	2.5 ± 0.2 (2.3- 2.7)	-
Spicule	-	18.3 ± 0.8 (17.4- 19)	-
Gubernaculum	-	4.5 ± 0.1 (4.4- 4.6)	-

*Measurements are in micrometer and proportion is expressed in percentage. mean ± sd (range), respectively.

F e m a l e : Body almost straight when killed by gentle heat. Cuticle with fine annulation. Lateral lines not clearly marked. Head wide, offset, without annulation. Stylet medium in size and robust, conus about 55 to 60 percent of stylet length, knobs rounded. Esophagus with relatively long procorpus, oval median bulb, short isthmus, and overlapping terminal bulb with long dorsal esophageal gland lobe. Excretory pore after median bulb, at the level of or posterior to nerve ring. Hemizonid situated posterior to excretory pore. Female reproductive system is monodelphic, outstretched. Oocytes mostly in a single row, spermatheca not offset, the post-vulval uterine sac is almost equal to body width at vulva. Vulva is situated at about 70 percent of body. Tail is sharp conical and mucronate (Fig. 1).

M a l e : Body ventrally curved when killed by heat, with sharp curve in tail region. Head, stylet, esophagus and excretory secretory system, are same as that female. Single

reflexed testis. Spicules are rose-thorn shape with *hook-shaped* distal tips. Gubernaculum-like structure is almost triangular. Bursa absent, caudal papillae in three pairs, all posterior to cloaca (Fig. 1).

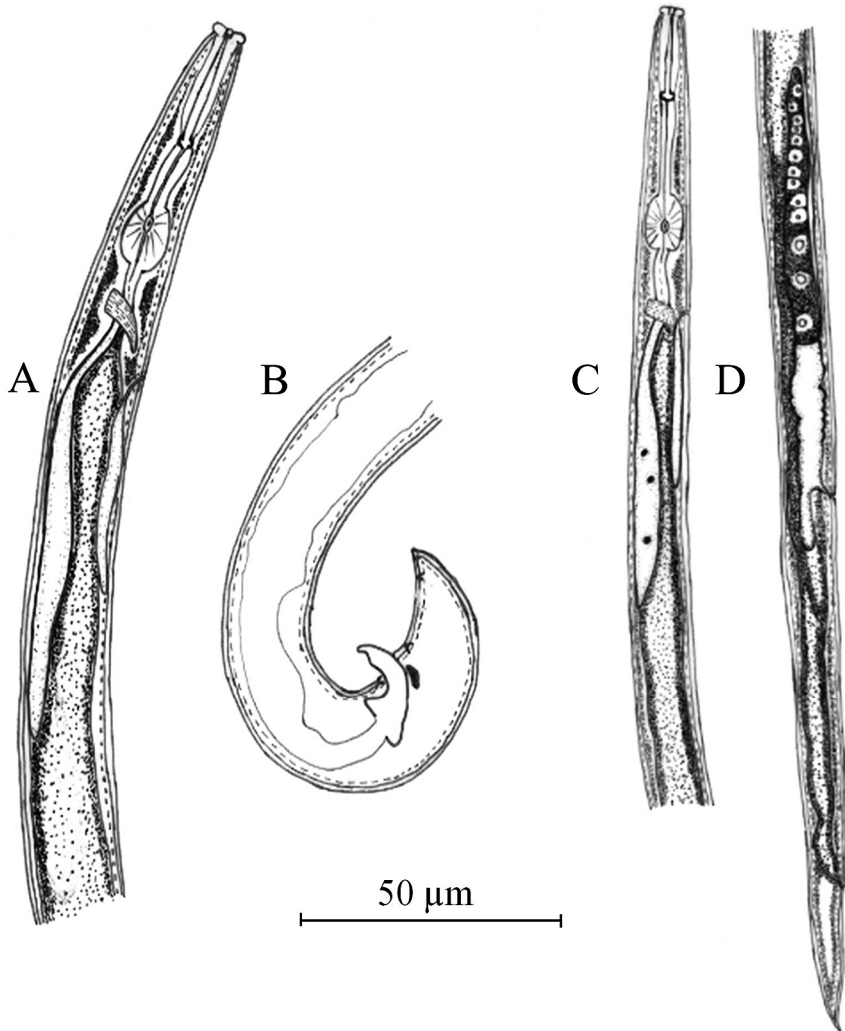


Fig. 1: Morphological characteristics of the *Schistonchus caprifici* isolated from caprifig syconium. A and B: male; C and D: female

Discussion: The materials examined here are agreed to description of VOVLAS & LARIZZA (1996). Because the excretory pore is posterior to median bulb, *S. caprifigi* is different from *S. altissimus*, *S. africanus*, *S. aureus*, *S. aculeata*, *S. altermacrophylla* and *S. aevigatus*, and is similar to *S. hispida*, *S. macrophylla*, *S. racemosa* (NICOLE & ROBIN 2001). In *S. racemosa*, there are two pairs of sexual papillae in male tail, spermatheca sphaerical and filled with rod-shaped sperm (REDDY & RAO 1986). In *S. hispida*, one pair of papillae are pre cloacal, one pair are close to cloaca, and one pair are located at tail tip, but in case of *S. caprifigi*, all of three pairs are post cloacal. Morphometrical differences between Iranian population and the population reported by VOVLAS & LARIZZA (1996), is given in Tab 3.

Results of study of population change: The results of population change of *S. caprifigi* in Estahban has been shown in Fig. 2. The highest mean of population density for male and females in spring caprifig was recorded as 515.5 and 751, respectively. Winter caprifig, with means of 260.25 male and 379.5 female nematodes, stands in second rank of population density

Tab 3: Morphometric comparison of *Schistonchus caprifigi* collected from Estahban and population collected by VOVLAS & LARIZZA (1996)

	a	b	b'	Stylet length	Tail length
Estahban region	28.8- 36.2	4.9- 5.8	2.8- 4.1	20- 22	30- 40
VOVLAS & LARIZZA (1996)	29.4- 40.2	5.7- 7.1	3.7- 5	22.1- 23.4	31.8- 44.8

Measurements are in micrometer and proportion is expressed in percentage.

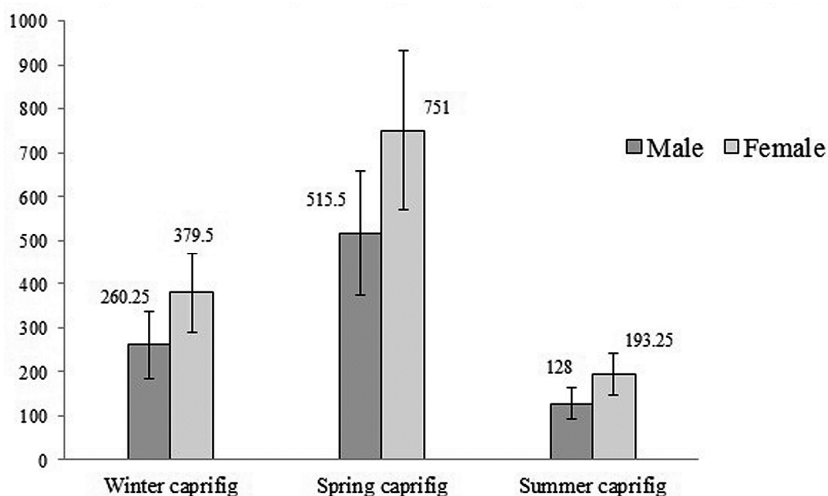


Fig. 2: Population change of *Schistonchus caprifigi* in winter, spring and summer caprifigs

S. africanus have been isolated from the fig pollinator wasp, *Elisabethiaal stuckenberg* (VOVLAS et al. 1998). According to their study, numbers of morphologically similar of this nematode were found in hemocoel of female wasps and florets tissues of fig, but not found in body of wingless male wasps. The relationship between *S. caprifici*, *B. psenes* and the cleptoparasite *P. caricae*, isolated from florets of fig syconium in spring, summer and winter seasons in Southern Italy have been studied by VOVLAS et al. (1996). In their investigation, all of life stages of this nematode was found in hemocoel of winged females of both species of wasp, but no relationship with wingless males was observed.

There is little information on population change of *S. caprifici*. Considering the importance of fig tree in Iran, PAKNIYAT & TAGHIZADEH (2000), has determined the average population density of *S. caprifici* in fruits of overwintering wild figs, in three major fig cultivation regions of Fars province, namely Estahban, Kazeroon and Absard, which were 660, 625 and 785, respectively. Based on our results, female-to-male sex ratio in winter, spring and fall caprifigs was estimated about 1.5:1 which was almost agree with the findings of DECRAPPEO & GIBLIN-DAVIS (2001) that was 2:1 in phases C and D of syconium.

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Authors' addresses:

Maryam KOLAEI

M.Sc. student of Agricultural Entomology, Department of Plant Protection, Faculty of Agriculture, Yasouj University, Yasouj, Iran
E-mail: Maryam.kolaei@yahoo.com.

Mohammad ABDOLLAHI

Associate professor of Nematology, Department of Plant Protection, Faculty of Agriculture, Yasouj University, Yasouj, Iran
E-mail: mdabdollahi@gmail.com, *Corresponding Author

Alireza MONFARED

Associate Professor of Agricultural Entomology, Department of Plant Protection, Faculty of Agriculture, Yasouj University, Yasouj, Iran
E-mail: Alirezamonfared1@yahoo.com.

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Maximilian SCHWARZ, Konsulent f. Wissenschaft der Oberösterreichischen Landesregierung, Eibenweg 6, A-4052 Ansfelden, Austria; maximilian.schwarz@liwest.at.

Redaktion: Fritz GUSENLEITNER, Biologiezentrum Linz, f.gusenleitner@landesmuseum.at;
Roland GERSTMEIER, Lehrstuhl f. Zoologie, TU München, gerstmei@wzw.tum.de;
Thomas WITT, Tengstraße 33, D-80796 München, thomas@witt-thomas.com;
Berthold CLEWING, Akademischer Verlag München, avm@druckmedien.de;
Harald SULAK, Museum Witt München, h.sulak@atelier-sulak.de.

Mitarbeiter: Karin TRAXLER, Biologiezentrum Linz, bio.redaktion@landesmuseum.at;
Heike REICHERT, Museum Witt München, heike_reichert66@web.de;
Erich DILLER, Zool. Staatssammlung München, erich.diller@zsm.mwn.de.

Adresse: Entomofauna, Redaktion und Schriftentausch Thomas WITT, c/o Museum Witt München, Tengstr. 33, 80796 München, Deutschland, thomas@witt-thomas.com;
Entomofauna, Redaktion c/o Fritz GUSENLEITNER, Lungitzerstr. 51, 4222 St. Georgen/Gusen, Austria, f.gusenleitner@landesmuseum.at.

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