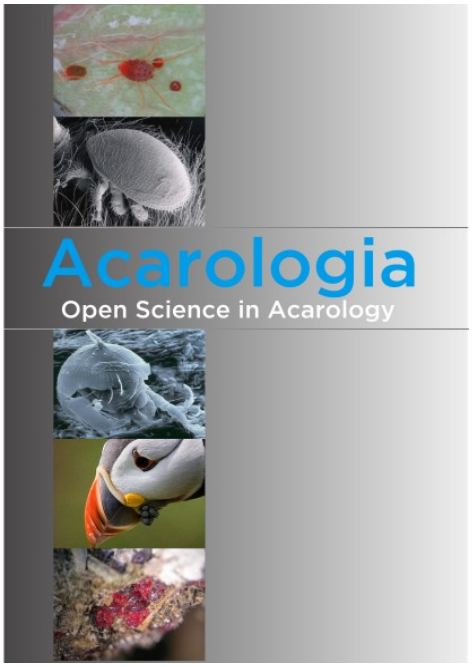

Acarologia

A quarterly journal of acarology, since 1959
Publishing on all aspects of the Acari

All information:
http://www1.montpellier.inra.fr/CBGP/acarologia/
acarologia-contact@supagro.fr

OPEN ACCESS

Acarologia is proudly non-profit,
with no page charges and free open access

Please help us maintain this system by
encouraging your institutes to subscribe to the print version of the journal
and by sending us your high quality research on the Acari.

Subscriptions: Year 2022 (Volume 61): 450 €
http://www1.montpellier.inra.fr/CBGP/acarologia/subscribe.php
Previous volumes (2010-2020): 250 € / year (4 issues)
Acarologia, CBGP, CS 30016, 34988 MONTFERRIER-sur-LEZ Cedex, France
ISSN 0044-586X (print), ISSN 2107-7207 (electronic)

The digitalization of Acarologia papers prior to 2000 was supported by Agropolis Fondation under the reference ID 1500-024 through the « Investissements d'avenir » programme (Labex Agro: ANR-10-LABX-0001-01)

INVESTISSEMENTS D'AVENIR

agropolis fondation
Supporting agricultural research for sustainable development

ON THE IDENTIFICATION OF A TAXA COLLECTED FROM EGYPT IN THE SPECIES SUB-GROUP *ANDERSONI*: MORPHOLOGICAL RELATIONSHIPS WITH RELATED SPECIES AND MOLECULAR ANALYSIS OF INTER AND INTRA-SPECIFIC VARIATIONS (ACARI: PHYTOSEIIDAE)

BY H. A. I. RAMADAN [1], E. M. EL-BANHAWY [2] & S. I. AFIA [3]

(Accepted July 2009)

MORPHOLOGY
GENETICS
IDENTIFICATION

SUMMARY: Morphological studies of the diagnostic characters of *Amblyseius swirskii* Athias — Henriot (original description) and *A. enab* El Badry (several new collections including the type locality) indicated close affinity between the two species. Among the Egyptian collections, little morphological variations have been observed except for setae *Z1*, *S2*, *S4* and *S5*.
Sequencing of a fragment of the internal transcribed spacer (ITS) region (ITS1, ITS2 and 5.8S) of different samples from Egypt showed almost complete identity with *Neoseiulus swirskii (= A. swirskii* Accession number EU 310505).
Other related species i.e. *A. cucumeris* (Oud.), *N. andersoni* (Chant) and *N. fallacis* (Garman) showed distant identity. The intraspecific variations were great between the six samples of Egypt and mainly because of mutations (which could be due to error during replication or as a result of mutagens).

INTRODUCTION

Molecular applications have had particular success in facilitating the identification of taxonomically difficult species, understanding population structures and elucidating phylogenetic relationships (BLACK & DUTEAU, 1997; NICOL *et al.*, 1997; PERROT-MINNOT & NAVAJAS, 1995).

NAVAJAS *et al*. (1992), found that two sympatric species of *Eotetranychus* morphologically very similar showed substantial genetic divergence. Similar pattern of variations have also been reported in the cassava green mite *Mononychellus progresivus* Doresta with intra-specific diversity being low for both ITS and Cytochrome Oxidase subunit 1 gene (Co1), yet lower in ITS2 (NAVAJAS *et al.* 1994).

NAVAJAS *et al.* (1999) compared the sequences of ITS1 ITS2 and 5.8S r DNA in several species of phytoseiids and concluded that ITS1 is more variable than ITS2 and also more difficult to align. The ITS1 was the only region that showed polymorphism within the species. The predacious mite *Amblysieus swirskii* Athias–Henriot has been described as a new species from Algeria (1962). The author as well

1. Department of Cell Biology, National Research Center, Dokki, Cairo, Egypt.
2. School of Biology sciences, University of Nairobi, Kenya P. O. Box 30197
3. Department of Plant Protection, National Research Center, Dokki, Cairo, Egypt

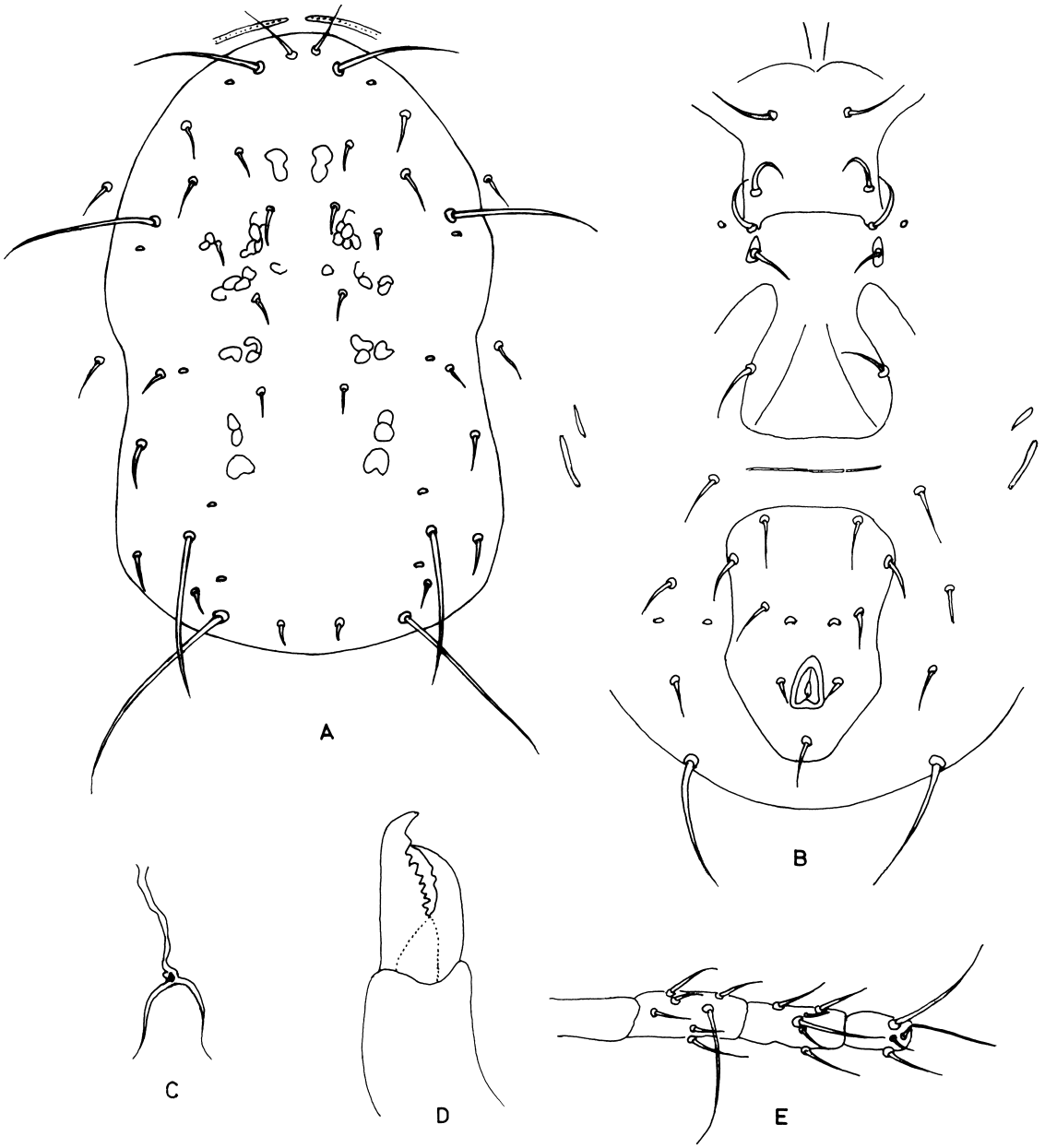

FIG. 1. — *Amblyseius swirskii* Athias- Henriot. A. — ♀ Dorsal shield. B. — Ventral shield. C. — Chelicerae. D. — Spermatheca. E. — Leg IV.

established the relationships with the related species *A. andersoni* (Chant). Later, the predacious mite *A. enab* El-Badry was described from Egypt; however, the morphological relationships with related species have not been established (El-Badry, 1967).

The *andersoni* species sub-group as defined by Chant & McMurtry (2004) contains 120 nominal species including *A. swirskii* and *A. enab*. In the present work morphological studies of the diagnostic characters of the included taxa were carried out on the original description of *A. swirskii* and the different collections from Egypt. To confirm the designation of taxa, additional studies were carried out to show inter and intra-specific sequence variations in a fragment of internal transcribed spacers (ITS1, ITS2 and 5.8S) region.

Materials and methods

Samples representing the type locality of *A. enab* and five different localities were collected, mounted in Hoyer's media and identified according to Athias-Henriot (1962), El-Badry (1967) and Chant & McMurtry (2004). Measurements are in microns (μ) and details of morphology were illustrated by using a phase — contrast microscope (40X). The fine details of spermatheca and chelicerae were identified by using oil immersion lens (100X).

Genomic DNA was extracted according to Navajas *et al.* (1999). Total DNA was precipitated with one volume iso-propanol, the pellet was washed with 75% ethanol and re-suspended in 200 l double distilled water. Fragments of the nuclear ribosomal transcribed spacers (ITS) region were PCR — amplified and sequenced. The ITS primers used were that described by Ramadan *et al* (2004). The amplified PCR products were purified and concentrated by using a centrifugal filter device (Centricon X 100, Millipore). Double way sequencing for the fragment was performed by using Big Dye Terminator cycle sequencing kit (Applied Biosystems, Fostercity CA, USA). Sequence products were resolved on an "Applied Biosystem ABI" Prism 310 automated DNA sequencing system. Direct submissions were made to National Center for Biotechnology Information (NCBI) GenBank database. The Egyptian *A. swirski* ITS sequences used in this study were deposited in GenBank database under Accession Numbers: EU924212- EU924217 (Ramadan *et al.* 2008). Other Phytoseiidae ITS sequences obtained from GenBank database were used for comparison with our data: *Neoseiulus swirskii* (EU310505), *Neoseiulus andersoni* (EU310504), *Neoseiulus cucumeris* (AY121985), *Iphiseius degenerans* (AY121984), *Euseius finlandicus* (AF209437), *Euseius finlandicus* (AF202993), *Neoseiulus californicus* (Y18269) and *N. fallacis* (Y18271).

Sequence analysis and multiple sequence alignment:

Pairwise sequence alignments were carried out using NCBI-BLASTN version 2.2.5 and PSI BLAST (Altschul *et al.* 1997). Multiple sequence alignments and variable sites extraction of ITS sequences from Egyptian and GenBank database samples were done using the MUSCLE 3.6 software (Edgar 2004) and CLUSTALW 1.82 (Thomson *et al.* 1994).

Results

The *andersoni* species- sub group is characterized by having female ventrianal shield longer than wide, ratio length to width 1.5: 1, setae z2, *z4,S2, S4, S5* short subequal; *Z4, Z5* elongate and spermatheca with bell shape caylx and ratio of length/ width at mid point of caylx < 3 : 1. Variations in the ratio of *z4*/*s4*, *s4*/ *Z1, s4*/*S2; Z4*/*Z5, j1*/*j3* differentiate species within the sub-group.

In the present work these diagnostic characters were almost the same in the included taxa indicating only one species i.e: *A. swirskii* (Table 1). However, there were some variations in length of setae *z4*, *S2* and *Z1*. The included species have been reported from many localities representing one geographical zone. (Mediterranean), it is accepted therefore, regardless the variations in some setae, that they are representing a single species (Fig. 1).

Samples examined from the type locality or else where in Egypt revealed morphological identity with some accepted variations in setae *S2*, *Z1*, *j1* and *j3* (Table1).

Ratio	(1)	(2)	(3)	(4)	(5)	(6)	*A. swirskii*
j1/j3	0.48	0.54	0.55	0.48	0.53	0.57	0.52
z4/s4	0.22	0.21	0.18	0.18	0.20	0.19	0.24
Z4/Z1	9.4	9.3	9.7	9.4	8.8	7.3	6.3
s4/s2	4.7	4.7	4.7	4.0	3.7	4.1	3.8
Z4/Z5	0.68	0.70	0.66	0.66	0.64	0.64	0.71
Ventrianal L/W	1.5	1.5	1.5	1.5	1.5	1.6	1.48

TABLE 1. — Diagnostic characters of *A. swirskii* sampled from citrus and grapes in the Nile delta of Egypt and the holotype (Athias-Henriot, 1962).

Sequencing of a fragment of ITS region (ITS1, ITS2, 5.8S) indicated almost complete identity of the Egyptian samples with *Neoseiulus swirskii,* accession number EU 310505 (= *A. swirskii*) (TABLE 2). Regardless the locality within Egypt, taxa were less identical when compared to the related species *N. cucumeris* (Oud.) *N. andersoni* (Chant), and *N. fallacis* (Garman). According to the molecular analysis (TABLE 3), samples are grouped into three groups: **Group 1** (sample 2 (EU924215), sample 3 (EU924213) and sample 4 (EU924214), **Group 2** (sample 1 (EU924212) and a sample from GenBank database (*Neoseiulus swirskii*, accession number EU310505) and **Group 3** (sample 5 (EU924216) and sample 6 (EU924217)

	Samples of *A. swirskii*																	
	(1)			**(2)**			**(3)**			**(4)**			**(5)**			**(6)**		
	L	S	ID	L	S	ID	L	S	ID	L	S	ID	L	S	ID	L	S	ID
N. swirskii	601	1340	98	601	1177	92	601	1131	90	601	1159	91	601	1247	94	601	1280	95
N. cucumeris	649	1277	95	649	1123	90	649	1048	86	649	1105	89	649	1210	92	649	1235	93
N. andersoni	555	1272	95	528	1123	90	555	1043	86	555	1100	89	555	1215	93	594	1235	93
N. fallacis	594	1268	95	594	1114	89	594	1039	86	594	1096	88	594	1211	92	555	1230	93

TABLE 2. — A comparison between (identity of the fragment of nuclear ITS region) some related species and samples of *A. swirskii* collected from citrus and grape orchards in the Nile delta of Egypt.
L= Length ; S = score ; **ID** = identity %

The variable sites between these groups were recorded inside the studied ITS region. We used the nucleotide numbers in one of our samples (sample 3, accession number EU924213) as a reference to identify the variable sites in other samples (TABLE 3).

Nucleotide number (Accession No. EU924213)			23	33	35	88	145	146	147	148	149	163	182	216	223	249	262	271	274	287	300	304	310-311	314	315	318-319	323	332
Sample	Accession Number	Phylogenetic Group	ITS1			5.8S rRNA													ITS2									
Sample 3	EU924213	Group 1	c	c	c	a	c	c	a	t	a	c	c	a	g	t	c	a	t	a	c	c	-	a	g	-	a	g
Sample 4	EU924214		c	c	c	a	c	c	a	t	a	c	c	a	g	t	c	a	t	a	c	c	-	a	g	-	a	g
Sample 2	EU924215		a	a	t	a	t	c	a	t	a	-	c	a	g	t	c	a	t	a	c	c	-	a	g	-	a	g
Sample 1	EU924212	Group 3	a	a	t	-	t	g	t	-	-	-	t	-	t	t	t	t	-	t	-	t	t	t	t	t	a	-
N. swirskii	EU310505		a	a	t	-	t	g	t	-	-	-	t	-	t	t	t	t	-	t	-	t	t	t	t	t	a	t
Sample 5	EU924216	Group 2	n	a	t	-	t	g	t	-	-	-	t	a	g	a	t	t	-	c	c	t	t	a	t	g	g	t
Sample 6	EU924217		n	n	n	-	t	g	t	-	-	-	t	a	g	a	t	a	-	c	-	t	t	a	t	-	g	t

TABLE 3. — The variable sites (a = Adenine, c = Cytosine, g = Guanine, t = Thymine, - = deletion and n = Not detected) detected in a fragment of nuclear ITS region of six samples of *A. swirskii* collected from citrus and grapes in the Nile delta of Egypt.

The variable sites between group one and other groups showed that there are twelve SNPs (single nucleotide polymorphism) sites, eight transversions and four transitions. Also, there are five indels (four insertions & one deletion).

The variable sites detected between group 2 and other groups showed (T-A, transversion) at position 314 and (T-G, transversion) at position 223. Also, insertion of T at position 318-319 and a deletion at position (216) were detected in case of group 2 only.

The variable sites detected between group 3 and other groups showed, (A-T, transversion) at position 249 and (G-A, transition) at position 323.

Discussion

The diagnostic characters of the Egyptian collection and the original description of *A. swirskii* were identical, yet, indicating a single taxa. Variations in length of some setae such as *S2* have been reported among population of *A. swirskii* in different localities within the Mediterranean (Chant & McMurtry, 2004). They have considered these populations representing a single species despite the presence of variations in some setae. We are in full agreement with this conclusion irrespective of the observed variations in some setae in the collections of *A. swirskii* from Egypt. Sequence of a fragment of ITS region (ITS1, ITS2 and 5.8S) of the different samples from Egypt showed identical alignment with *N. swirskii* (= *A. swirskii*) and confirming the agreed morphology with the original description.

Usually the closely related species show interspecific variations despite that they are morphologically identical.

The two sympatric species of *Eotetranychus* which are morphologically identical showed substantial molecular divergence (Navajas *et al.* 1992). Likewise, the sequence of ITS1, in several phytoseiid species showed great variations between the included species (Navajas *et al.,* 1999). In the present work, the studied sequence of ITS region (ITS1, ITS2, 5.8S), in *A. swirskii* were distant from that of the closely related species in the species -subgroup, *andersoni.* In contrast, in polymorphic species like *A. swirskii* sequences of the internal transcribed spacers may not be useful to establish phylogenies within populations of that species. Populations are usually subject of mutations (which could be due to error made during replications or the action of chemical or physical mutagens) particularly in the natural fast evolving parts.

References

Altschul (S.F.), Stephen (F.), Maden (T.L.), Schaffer (A.A.), Zhang (J.), zhang (Z.), Miller (W.) & Lipman (D.J.), 1997. — Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. — Nucleic Acids Res. 25: 3389-3402.

Athias-Henriot (C.), 1962. — *Amblyseius swirskii*, a new phytoseiid related to *A. andersoni* (anactinotrichid mites). — Ann. Ecole. Nat. Agric. Alger 3 (5): 1-7.

Black (W.C.), & Duteau (N.M.), 1997. — RAPD-PCR and SSCP analysis for insect population genetic studies. In: Molecular Biology of insect Disease vectors: A methods manual, J.M. Crampton, C.B.Beard and C. Louis (eds.), — Chapman and Hall, ISBN 0412736608.

Chant (D.A.) & McMurtry (J.A.), 2004. — A review of the subfamily Amblyseiinae Muma (Acari: Phytoseiidae): Part III. The Tribe Amblyseiini Wainsten, Sub tribe Amblyseiina N. Sub tribe. — Internat. J. Acarol., 30 (3): 171-228.

Edgar (R.C.), 2004. — MUSCLE: Multiple sequence alignment with high accuracy and high throughput. — Nucl Acids Res 32: 1792-1797.

El-Badry (E.A.), 1967. — Five new phytoseiid mites from UAR, with collection notes on three other species (Acarina: Phytoseiidae). — Indian J. Ent. 29 (2): 177-184.

Navajas (M.), Cotton (D.), Kreiter (S.), Gutierrez (J.), 1992. — Molecular approach in spider mites (Acari: Tetranychidae): preliminary data on ribosomal DNA sequences. — Exp. Appl. Acarol. 15: 211-218

Navajas (M.), Gutierrez (J.), Bonato (O.), Bolland (H.R.) & Mapangon-Divassa (S.), 1994. — Intraspecific diversity of the cassava Green mite (Acari: Tetranchidae) using comparison of mitochondrial and nuclear ribosomal DNA sequences and cross-breeding. — Exp. Appl. Acarol. 18: 351-360.

Navajas (M.), Lagnel (J.), Fauvel (G.) & De Moraes (G.), 1999. — Sequence variation of ribosomal internal transcribed spacers (ITS) in commercially important phytoseiidae mites. — Exp. Appl. Acarol. 23: 851-859.

Nicol (D.), Amstrong (K.F.), Wratten (S.D.), Cameron (C.M.), Frampton (C.) & Fenton (B.), 1997. — Genetic variation in an introduced aphid pest *(Metopolophium dirhodum)* in New Zealand and relation to individuals from Europe. — Mol. Ecol. 6: 255-265.

Perrot-Minnot (M.J.) & Navajas (M.), 1995. — Bi parential inheritance of RAPD markers in males of the pseudo-arrhenotokous mite *Typhlodromus pyri*. — Genome 38: 838-844.

Ramadan (H.A.I.), El-Banhawy (E.M.), Hassan (A.A.), & Afia (S.I.), 2004. — Genetic variation in the predacious phytoseiid mite, *Amblyseius swirskii* (Acari: Phytoseiidae): Analysis of specific mitochondrial and nuclear sequences. — Arab J. Biotech. 7 (2): 189-196.

Ramadan (H.A.I.), El-Banhawy (E.M.), & Afia (S.I.), 2008. — Direct submission of Egyptian *Amblyseius swirskii* ITS sequence data to: National Center for Biotechnology Information (NCBI) GenBank Database. Accession Numbers: EU924212-EU924217

Thompson (J.D), Higgins (D.G), & Gibson (T.J.), 1994. — CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. — Nucleic Acids Res. 22: 4673-4680.