On the phylogenetic relationships of *Scaptodrosophila rufifrons* and *S. lebanonensis* (Diptera, Drosophilidae)

GERHARD BÄCHLI[1], ELISABETH HARING[2] & CARLOS R. VILELA[3]

[1] Zoologisches Museum, Universität Zürich, Winterthurerstrasse 190, CH-8057, Zürich, Switzerland.
    E-mail: baechli@zoolmus.unizh.ch
[2] Naturhistorisches Museum Wien, 1. Zoologische Abteilung, Burgring 7, A-1010 Wien, Austria.
    E-mail: Elisabeth.Haring@univie.ac.at
[3] Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo,
    Caixa Postal 11461, São Paulo - SP, 05422-970, Brazil.
    E-mail: crvilela@ib.usp.br

Six species of the *victoria* and *rufifrons* groups of *Scaptodrosophila* have been analyzed by DNA sequence analyses [nuclear and mitochondrial DNA]. The *rufifrons* group was shown to be monophyletic, containing two clades identical with the species *S. lebanonensis* and *S. rufifrons* respectively. The taxa *S. pattersoni*, *S. stonei*, and *S. galloi* are proposed as junior synonyms of *S. lebanonensis*. A proposal is also made to remove *S. deflexa* from the *victoria* group and to transfer the remaining species *S. brooksae*, *S. ebonata* and *S. throckmortoni* from the *victoria* to the *rufifrons* species group. Accordingly, the *victoria* group becomes a monotypic group, containing solely *S. victoria*, whose identity remains uncertain. A redescription of *S. lebanonensis* is included.

INTRODUCTION

Scaptodrosophila with more than 300 described species is one of the largest genera in the family Drosophilidae. An overview was given by Bock & Parsons (1978), who listed 11 species groups, but left many species ungrouped. Main characters of this genus are the presence of a median katepisternal seta which is not much shorter than the anterior one, and the presence of one pair of elongated setae in front of the scutellum, usually called prescutellar setae. It is known, however, that many species were included in this heterogeneous taxon, although the common discriminative characters are either missing or their presence appears at least doubtful. There is no comprehensive revision of *Scaptodrosophila* available and one may suppose that the genus in its currently accepted boundaries is not monophyletic.

While checking the male terminalia of several European specimens considered to be either *Scaptodrosophila rufifrons* (Loew, 1873) or *S. lebanonensis* (Wheeler, 1949), we (GB, CV) realized that the characters used to discriminate between these two sibling species are very variable and in general do not allow a clear-cut identification (Bächli *et al.* 2004). On the other hand, it was clear that two cryptic species must be involved which differ in their ecological preferences. One, traditionally named *S. rufifrons*, is collected in woodland areas and therefore considered a wild species. The other one, called *S. lebanonensis*, is predominantly found in wine cellars in warm, wine producing areas, thus representing a domestic species.

Cultivation of the wild species in the laboratory has proved to be difficult (Basden 1954), whereas strains of the domestic species have been kept for more than 50 years in the USA, originally in the «National *Drosophila* Species Resource Center» at Austin, Texas, now in «The Tucson *Drosophila* Species Stock Center» at Tucson, Arizona. *S. lebanonensis* has been used as a laboratory animal in many studies of phylogeny (e.g. Kwiatowski & Ayala 1999; Katoh *et al.* 2000; Tarrio *et al.* 2001), evolution (e.g. Pitnick *et al.* 2000), physiology (e.g. van Herrewege & David 1997) and many other subjects.

In his study of the *Drosophila* subgenus *Pholadoris* (now genus *Scaptodrosophila*), Wheeler (1949: 143) described the species *S. lebanonensis* based on a strain originated from flies collected by Sarah Bedicheck Pipkin in Beirut, Lebanon. In the same paper, he established the *victoria* species group, comprising *S. victoria* Sturtevant, 1942, *S. coracina* Kikkawa and Peng, 1938 (now member of the *coracina* species group, see Mather 1955), *S. nitens* Buzzati-Traverso, 1943 (now *S. rufifrons* Loew, see Papp *et al.* 1999), and *S. lebanonensis*. Descriptions and illustrations of the external male terminalia of *S. victoria*, *S. coracina*, and *S. lebanonensis* were provided by Hsu (1949: 128). It should be pointed out that, later on, Wheeler himself (Wheeler 1959: 192) considered *D. lebanonensis* as a probable synonym of *D. victoria*.

In the following years, additional species of the *victoria* group were described: *S. pattersoni* and *S. stonei*, both based on strains originated from flies collected in Lebanon (Pipkin 1956), *S. brooksae* and *S. lebanonensis casteeli*, both based on strains from Arizona (Pipkin 1961), *S. ebonata* (Parshad & Duggal 1966) from India, *S. throckmortoni* (Okada 1973) from Japan, and *S. galloi* (Lourenço & Mourão 1992) from Brazil. Herting (1955) also included the European *S. deflexa* (Duda 1924) in the *victoria* group. An additional, but undescribed possible member was reported by Imasheva *et al.* (1994) from the Caucasus area. Finally, the European species were taxonomically revised by Papp *et al.* (1999) who established the new *rufifrons* species group comprising the following five nominal species: *S. rufifrons*, *S. lebanonensis*, *S. pattersoni*, *S. stonei*, and *S. abdita* Papp, Rácz and Bächli. Therefore, the species remaining in the *victoria* group were *S. brooksae*, *S. deflexa*, *S. ebonata*, *S. galloi*, *S. throckmortoni*, and *S. victoria*.

Numerous publications are dealing with *S. rufifrons* and particularly with *S. lebanonensis* (see Papp *et al.* 1999). On the other hand, there are only few papers on the other taxa, e.g., for *S. galloi* (sometimes under the manuscript name *S. dimorpha*): Beverley & Wilson (1982), Daniels *et al.* (1990), Pelandakis & Solignac (1993), Blesa *et al.* (2001); for *S. pattersoni* and *S. stonei*: Clayton & Wheeler (1975), Máca (1988), Daniels *et al.* (1990), Papp *et al.* (1999); for *S. lebanonensis casteeli*: Clayton & Wheeler (1975), Fauron & Wolstenholm (1976), Throckmorton (1982), Daniels *et al.* (1990).

The reproductive barriers between certain *victoria* group species were studied by Pipkin (1961), using crossing experiments. In that publication as well as in papers published later (Pipkin 1962, 1963, 1965), it was stressed that the species studied show a considerable color variation; the genetic background of the color polymorphism in *S. lebanonensis* was later analyzed by Pipkin (1965). We want to emphasize here that, considering the actual knowledge about sterility problems in many *Drosophila* species (see Ashburner *et al.* 2005), the interpretation of the crossing experiments presented by Pipkin leave some doubts regarding the status of the *vic-

toria group species studied by her. Furthermore, the descriptive data she provided, in particular the details of the male terminalia, proved to be almost useless for species discrimination (see below).

As a consequence, we performed a phylogenetic analysis based on DNA sequences to clarify the relationships of the two cryptic species *S. rufifrons* and *S. lebanonensis* as well as of some related taxa. We hypothesize that the two species just mentioned, which are certainly different in their ecological background, represent good species.

MATERIAL AND METHODS

We investigated 16 specimens of *Scaptodrosophila* species representing the following taxa: *S. lebanonensis*, *S. lebanonensis casteeli*, *S. rufifrons*, *S. pattersoni*, *S. stonei*, *S. galloi*, (all members of the *rufifrons* group), *S. deflexa* (*victoria* group), *S. paratriangulata* Gupta & Ray-Chaudhuri, 1970 (ungrouped). All specimens had been kept in 70 % alcohol plus 5 % glycerin for some time. There are some doubts about the identity of the strain of *S. stonei*, originating from Teheran, Iran, as the original strain of this species was lost (Pipkin 1961). The strain originating from Veyo is interpreted by us as belonging to the subspecies *S. lebanonensis casteeli*, as mentioned by Pipkin (1961). Outgroup taxa used are *D. melanogaster* Meigen, 1830 and *D. simulans* Sturtevant, 1919. Sample codes, collecting localities, source, and GenBank accession numbers are listed in Tab. 1.

DNA extraction and PCR amplification

DNA was extracted from single flies preserved in 70 % ethanol plus 5 % glycerin by incubation in 300 µl of a 10 % Chelex (Biorad) solution containing proteinase K (0.5 mg/ml). After incubation (4 h, 56 °C, with agitation) solutions were heated to 95 °C for 5 min and centrifuged for 1 min. For purification and to remove short fragments of degraded DNA the supernatant was purified using the QIA Quick PCR Purification Kit (QIAGEN) with a final volume of 30–70 µl elution buffer. 3–7 µl of the DNA solution were used for the PCR. Control extractions without tissue were prepared for the PCR experiments. PCR was performed with an Eppendorf Thermocycler in a volume of 25 µl, containing 1 unit Dynazyme DNA polymerase (Finnzymes OY), 0.5 µM of each primer, and 0.2 mM of each dNTP. The solutions were heated to 95 °C (2 min) and then put through 30 reaction cycles: 95 °C (10 s), 54 °C (10 s), 72 °C (20 s), followed by a final extension at 72 °C (5 min). Negative controls for PCR reactions were performed to screen for contaminated reagents: i) control extractions (without DNA) instead of template; ii) reaction with water instead of template. Partial sequences from two genes were amplified. The nuclear *alcohol dehydrogenase* gene (*Adh*) and the mitochondrial *cytochrome c oxidase* subunit 3 gene (*Cox3*). The following primers (Hagemann *et al.* 1996) were used to amplify a section of about 480 bp (depending on the length of the intron) of the *Adh* gene spanning the region exon2-intron2-exon3: Adh-e2+ (CTGGACTTCTGGGACAAGCG); Adh-e3– (TAGATGCCCGAGTCCCAGTG). For the *Cox3* sequences the following primers (Haring & Aspöck 2004) were used to amplify a 712-bp fragment: cox3-fwd (TAGTTGATTATAGACCATGACC); cox3-rev (ACATCAACAAAATGTCAATATCA).

PCR products were extracted from agarose gels using the QIA Quick Gel

Genus / Species	Code	Locality	Source	Cox3	Adh	GenBank (*Cox3, Adh*)
Scaptodrosophila						
lebanonensis lebanonensis	leb-1	Bordils, Spain	L. Serra	+	−	DQ155681
	leb-2	Beirut, Lebanon	11010-0021.0	+	+	DQ155682, DQ155674
lebanonensis casteeli	leb-3	Veyo, Utah, USA	11010-0011.0	+	−	DQ155683
pattersoni	pat-1	Beirut, Lebanon	11010-0031.0	+	+	DQ155680, DQ155671
galloi	gal-1	near Porto Alegre, RS, Brazil	11020-0051.0	+	+	DQ155679, DQ155673
stonei	sto-1	Teheran, Iran	11010-0041.0	+	+	DQ155677, DQ155672
	sto-2	Teheran, Iran	11010-0041.1	+	−	DQ155678
rufifrons	ruf-1	Dietikon ZH, Switzerland	G. Bächli	+	+	DQ155684, DQ155668
	ruf-2	Visp VS, Switzerland	G. Bächli	+	+	DQ155685, DQ155669
	ruf-3	Yazir, Turkey	R. Allemand	+	−	DQ155686
	ruf-4	Yazir, Turkey	R. Allemand	+	−	DQ155687
	ruf-5	Yazir, Turkey	R. Allemand	+	−	DQ155688
	ruf-6	Karaovabeli, Turkey	R. Allemand	+	−	DQ155689
	ruf-7	Pfynwald VS, Switzerland	G. Bächli	+	+	DQ155690, DQ155670
deflexa	def-1	Zürich, Switzerland	G. Bächli	+	+	DQ155676, DQ155667
paratriangulata	par-1	Kanha National Park, India	G. Bächli	+	−	DQ155675
Drosophila						
melanogaster	mel	—	Begun et al., 1999; Lewis et al., 1995	+	+	AF175215, U37541
simulans	sim	—	McDonald and Kreitman, 1991; J.W.O. Ballard, direct submission	+	+	X57361, AF200854

Extraction Kit (QIAGEN) and cloned (TOPO TA Cloning Kit, Invitrogen). Sequencing (both directions) was performed by MWG-Biotech (Ebersberg, Germany). Published *Adh* and *Cox3* sequences of *D. melanogaster* (*Adh*: AF175215, Begun *et al.* 1999; *Cox3*: U37541, Lewis *et al.* 1995) and *D. simulans* (*Adh*: X57361, McDonald & Kreitman 1991; *Cox3*: AF200854, J.W.O. Ballard, direct submission) were used as outgroup sequences.

Sequence analysis

Alignments and editing were performed with the software BioEdit version 5.0.9 (Hall 1999). No gaps or stop codons were present in the coding regions. Intron sequences of the *Adh* gene were removed for the phylogenetic analyses. To infer the phylogenetic relationships, distance based (NJ, neighbour-joining algorithm; Saitou & Nei 1987), and maximum parsimony (MP) algorithms were employed using PAUP (version 4.0b10; Swofford 2002). MP trees for *Cox3* were calculated using heuristic search with the TBR (tree bisection reconnection) branch swapping algorithm with a random taxon addition sequence (1000 replicates) and with exhaustive search for the *Adh* gene. The DELTRAN option (delayed character transformation) was used. The robustness of trees was tested by bootstrapping (1000 replications), with the TBR (tree bisection reconnection) branch swapping algorithm and a random taxon addition sequence (10 replicates). P-distances were used for the NJ tree. Applying other models for the computation of distances did not alter the tree topology.

RESULTS

The alignment of *Cox3* sequences obtained from 16 individuals has a length of 667 sites. In the NJ tree based on this alignment (Fig. 1) the basal node separates *S. paratriangulata* (ungrouped *Scaptodrosophila* species) from the rest, followed by *S. deflexa*, a species currently, but in our opinion inappropriately, considered to belong to the *victoria* group. The remaining sequences are grouped in two sister clades: one contains all *S. rufifrons* individuals (*rufifrons* clade) and the other one the remaining sequences comprising *S. lebanonensis*, *S. pattersoni*, *S. stonei*, and *S. galloi* (*lebanonensis* clade).

The *lebanonensis* clade consists of individuals from very distinct geographic regions, i.e., Iran, Lebanon, Spain, Brazil, and USA (Utah). The *rufifrons* clade is further divided into two subclades comprising specimens from Turkey and Switzerland, respectively. Yet, these two subclades are not well supported in the bootstrap analysis. Distances (Tab. 2) within the *lebanonensis* (0–0.9 %) and *rufifrons* (0.2–1.9 %) clades are rather low compared to those found between these two clades (7.5–8.8 %). The other two members of the subgenus *Scaptodrosophila* (*S. deflexa*, *S. paratriangulata*) are only distantly related (12.0–15.0 %).

In the MP analysis eight shortest trees were obtained (TL = 256, CI = 0.789, RI = 0.878, RC = 0.693) which have the same topology as the NJ tree with the exception of the branching pattern within the *rufifrons* clade: The two subclades (Turkey / Switzerland), which would suggest a geographic differentiation, appear in only one out of the eight trees.

Adh sequences were obtained from *S. deflexa*, three representatives of the *rufifrons* clade, and four of the *lebanonensis* clade. From *S. paratriangulata* the *Adh*

Tab. 2. P-distance matrix of *Cox3* (below diagonal) and *Adh* (above diagonal) sequences. Abbreviations are according to Tab. 1.

	leb-1	leb-2	leb3	pat-1	gal-1	sto-1	sto-2	ruf-1	ruf-2	ruf-3	ruf-4	ruf-5	ruf-6	ruf-7	def-1	par-1	mel	sim
leb-1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
leb-2	0.3	—	—	0.0	0.3	0.8	—	5.7	5.9	—	—	—	—	5.7	18.6	—	22.2	21.6
leb-3	0.5	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
pat-1	0.5	0.2	0.0	—	0.3	0.8	—	5.7	5.9	—	—	—	—	5.7	18.6	—	22.2	21.6
gal-1	0.3	0.0	0.2	0.2	—	1.1	—	5.9	6.2	—	—	—	—	5.9	18.9	—	22.4	21.9
sto-1	0.9	0.6	0.8	0.8	0.6	—	—	6.5	6.8	—	—	—	—	6.5	18.6	—	22.7	22.2
sto-2	0.8	0.5	0.6	0.6	0.5	0.2	—	—	—	—	—	—	—	—	—	—	—	—
ruf-1	8.1	7.9	7.8	7.8	7.9	8.5	8.4	—	0.8	—	—	—	—	0.0	15.9	—	21.6	21.1
ruf-2	8.4	8.2	8.1	8.1	8.2	8.8	8.7	0.6	—	—	—	—	—	0.8	16.2	—	21.9	21.4
ruf-3	8.1	7.9	7.8	7.8	7.9	8.5	8.4	1.5	1.8	—	—	—	—	—	—	—	—	—
ruf-4	7.9	7.8	7.6	7.6	7.8	8.4	8.2	1.0	1.3	1.0	—	—	—	—	—	—	—	—
ruf-5	7.8	7.6	7.5	7.5	7.7	8,2	8.1	0.9	1.2	0.9	0.2	—	—	—	—	—	—	—
ruf-6	7.9	7.8	7.6	7.6	7.8	8.4	8.2	1.6	1.9	1.3	0.9	0.8	—	—	—	—	—	—
ruf-7	7.9	7.8	7.6	7.6	7.8	8.4	8.2	0.2	0.5	1.3	0.9	0.8	1.5	—	15.9	—	21.6	21.1
def-1	12.9	12.6	12.4	12.0	12.6	12.0	12.1	12.0	12.0	12.1	12.4	12.9	12.7	12.6	—	—	22.2	21.1
par-1	14.2	14.4	14.2	12.9	13.2	13.2	12.7	12.6	13.0	12.7	14.2	15.0	14.8	14.4	13.3	—	—	—
mel	13.9	13.8	13.6	11.8	11.8	12.0	11.8	11.7	11.8	11.7	13.6	14.1	13.9	13.8	12.4	12.1	—	1.9
sim	13.2	13.0	12.9	11.4	11.4	11.8	11.4	11.2	11.4	11.2	12.9	13.6	13.5	13.0	12.4	11.7	4.9	—
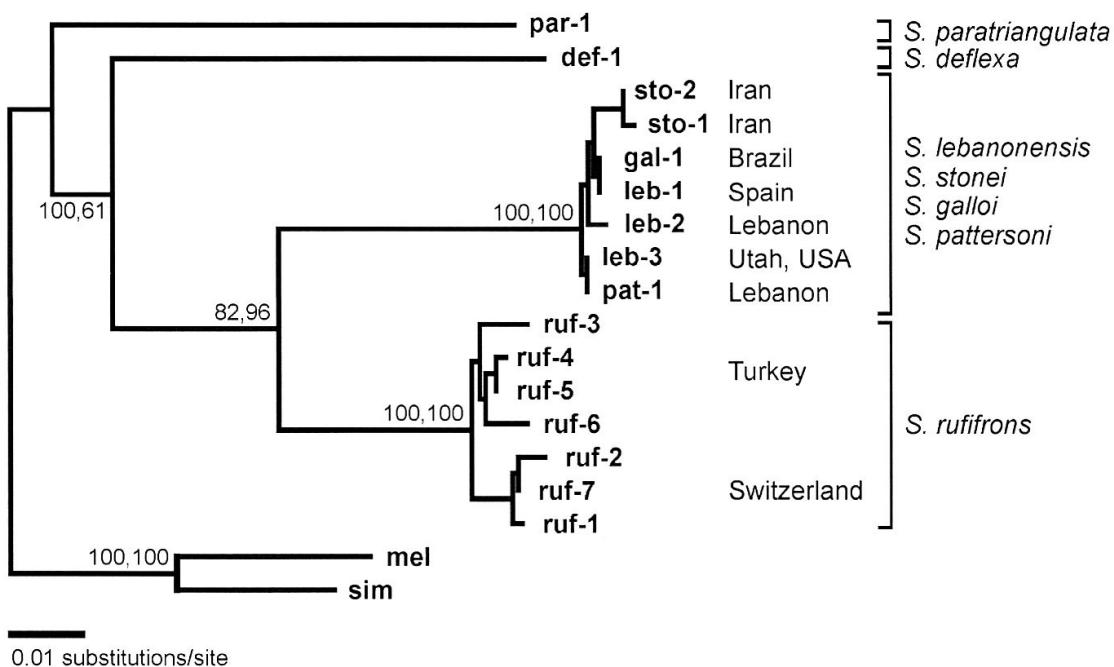
Fig. 1. *Cox3* sequences: NJ tree of 16 *Scaptodrosophila* specimens and two outgroup species (*D. melanogaster, D. simulans*). Abbreviations of specimens are according to Tab. 1. Taxonomic assignment and geographic origin are depicted on the right. Bootstrap values are given at the nodes (left: NJ, right: MP), except for internal nodes within the *rufifrons* and *lebanonensis* clades.
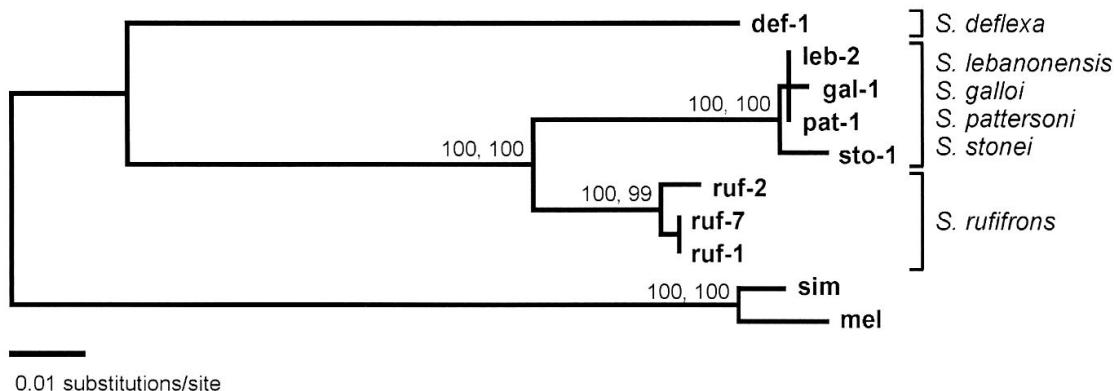
Fig. 2. *Adh* sequences: NJ tree of eight *Scaptodrosophila* specimens and two outgroup species (*D. melanogaster, D. simulans*). Abbreviations of specimens are according to Tab. 1. Taxonomic assignment and geographic origin are depicted on the right. Bootstrap values are given at the nodes (left: NJ, right: MP), except for internal nodes within the *rufifrons* and *lebanonensis* clades.

fragment could not be amplified (the DNA of this comparatively old sample was apparently not well preserved). The alignment (without intron) has a length of 370 bp. In both the NJ tree (Fig. 2) and the MP tree (one shortest tree, not shown; TL = 140, CI = 0.957, RI = 0.959, RC = 0.918) the topology is congruent with that of the *Cox3* tree. As can be seen from the distance matrix (Tab. 2), distances between the *lebanonensis* and *rufifrons* clades (5.7–6.8 %) are slightly lower compared to the

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Cox3 sequences. On the other hand, distances between the two clades and the other taxa are considerably higher. E.g., distances between ingroup and outgroup (*D. melanogaster* and *D. simulans*) range from 11.2–14.1 % in *Cox3* and from 21.1–22.7 % in *Adh*. This finding indicates that the *Cox3* gene reaches substitutional saturation much earlier (at a p–distance of about 11 %) than the *Adh* gene.

DISCUSSION

The results obtained with the two marker sequences *Cox3* and *Adh* are congruent and suggest the following taxonomic grouping: In spite of the problems in discriminating *S. rufifrons* from *S. lebanonensis* by means of morphological characters, the two species appear genetically well separated, corroborating their status as distinct species. This is in accordance with their apparent ecological adaptations. The *rufifrons* clade comprises only samples of *S. rufifrons*. The *lebanonensis* clade consists of four nominal species which are genetically very similar. Some individuals from different nominal species (originating from the Old and New World) are identical in the DNA sequences analysed. Thus, it seems likely that the presence of this group in the New World is the result of recent introduction, most probably due to human activities. Accordingly, their species status has to be revised.

The *rufifrons* group as previously defined by Papp *et al.* (1999) represents a monophylum in the DNA based trees, and, in contrast to the former classification, we think that the group consists of two species only, until more information is available on the status of the third species, *S. abdita*.

Since the publications of Pipkin, the species related to *S. lebanonensis* were taxonomically almost overlooked (but see the paper of Papp *et al.* 1999); there is only one publication (Máca 1988) dealing with specimens collected in Central Asia. The key for the identification of *S. lebanonensis*, *S. pattersoni*, and *S. stonei* provided by Máca (1988) included mainly color characters and is probably based on the original descriptions by Pipkin. However, it should be pointed out that the original illustrations of the male terminalia of *S. pattersoni* and *S. rufifrons* provided by Máca (1988: 9, figs. 10 and 11 respectively) fully agree with our current concept of *S. lebanonensis* and *S. rufifrons* respectively, but are the opposite regarding those illustrated by Papp *et al.* (1999).

The descriptions of *S. pattersoni* and *S. stonei* were based on morphological characters, such as differences in the male terminalia, but also on crossing experiments including *S. lebanonensis* (Pipkin 1956). It was stated that all species studied were highly variable particularly in color characters of the thorax, in the number of certain setae and egg filaments. Unfortunately, the drawings of the terminalia are of low quality. The material used was collected in the Lebanon years ago and has been kept as laboratory strains since then. No attempt was made by Pipkin to verify the constancy of the differences in the male terminalia. The main specific differences mentioned by her were the numbers of certain setae. However, according to our findings, their ranges overlap (Fig. 4).

The same story can be told about the background of the descriptions of *S. brooksae* and *S. lebanonensis casteeli* (Pipkin 1961). The strains used were collected in the western USA. The differences found among them and the other related species were again not substantial, particularly the strain of *S. lebanonensis casteeli* was almost identical with the original strain of *S. lebanonensis*. However, as the latter species was known at that time only from Lebanon, the establishment of a sub-

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species for the fully isolated population in Arizona seemed reasonable.

The original distribution of *S. lebanonensis*, as far as it is presently known, extends from the Mediterranean area in the West to Central Asia in the East, and is associated with a generally warm and dry climate. Although we do not have any data it can be assumed that *S. lebanonensis* was introduced to the New World (USA, Brazil) by humans. A transfer in connection with wine production seems plausible and even multiple invasions may have occurred. As collecting of drosophilids is a worldwide task, the discovery of *S. lebanonensis* in additional areas can be expected.

Unfortunately, no suitable specimens were available for the phylogenetic analysis of the remaining species of the *victoria* and *rufifrons* groups. However, we think that the status of the *rufifrons* species group (Papp *et al.* 1999) as a monophyletic lineage is corroborated. The characters of the male terminalia as well as our results of the sequence analyses show that *S. galloi* (called *S. dimorpha* in the National *Drosophila* Resource Center) does not deserve species status and has to be synonymyzed with *S. lebanonensis*. The species *S. abdita* clearly belongs to this group, as the characters of the male terminalia are not substantially different from those of *S. rufifrons* and *S. lebanonensis*. One might suppose that *S. brooksae*, whose original strain has been lost, also belongs to the *rufifrons* group and even could be a synonym to *S. lebanonensis*, considering that Pipkin (1961) described it as closely related with *S. lebanonensis casteeli*.

Additionally, based on the male terminalia drawings published by Okada (1973: 437), we suspect that on the one hand *S. throckmortoni* (as depicted in his figs 3A–C) could be a synonym of *S. rufifrons* and that on the other hand the Italian specimen named *S. rufifrons* (as depicted in his figs 3D–F) most probably belongs to *S. lebanonensis*. The internal male terminalia of *S. ebonata*, as illustrated in its original description (Parshad & Duggal 1966: 281, fig. 3), were most probably damaged. However, based on some details such as aedeagus' structures and paraphyses' shapes, it seems to belong to the *rufifrons* group as well.

The densely scaled outer paraphyses and distally microtrichose hypandrium, in addition to the trifurcate condition of inner paraphyses, morphologically place *S. deflexa* far from the remaining species ascribed to the *rufifrons* group. Furthermore, *S. deflexa* is also clearly separated from the remaining analyzed species regarding DNA analyses (Figs. 1–2). So, by combining morphological and molecular data *S. deflexa* must remain ungrouped within the genus *Scaptodrosophila*, even though it has recently been considered a member of the *victoria* group (see Bächli *et al.* 2004); the *victoria* group diagnosis given there is probably valid only for *S. deflexa* itself.

Thus, the *S. victoria* species group becomes a monotypic group; and assuming that the type series of *S. victoria* is lost, its identity will remain uncertain.

Full redescriptions of *S. rufifrons* and *S. deflexa* were published by Bächli *et al.* (2004). Here, we take the occasion to provide a redescription of *S. lebanonensis*.

Scaptodrosophila lebanonensis (Wheeler, 1949)

(Figs 3, 4A)

Diagnosis. Frons almost equal in length and width; all orbital setae in a row; orbital plates reddish, not silvery; decasternum with distal margin notched; hypandrium with more than 7 setae on each side; aedeagus membranous, globous and completely embraced by hypandrium.

lebanonensis
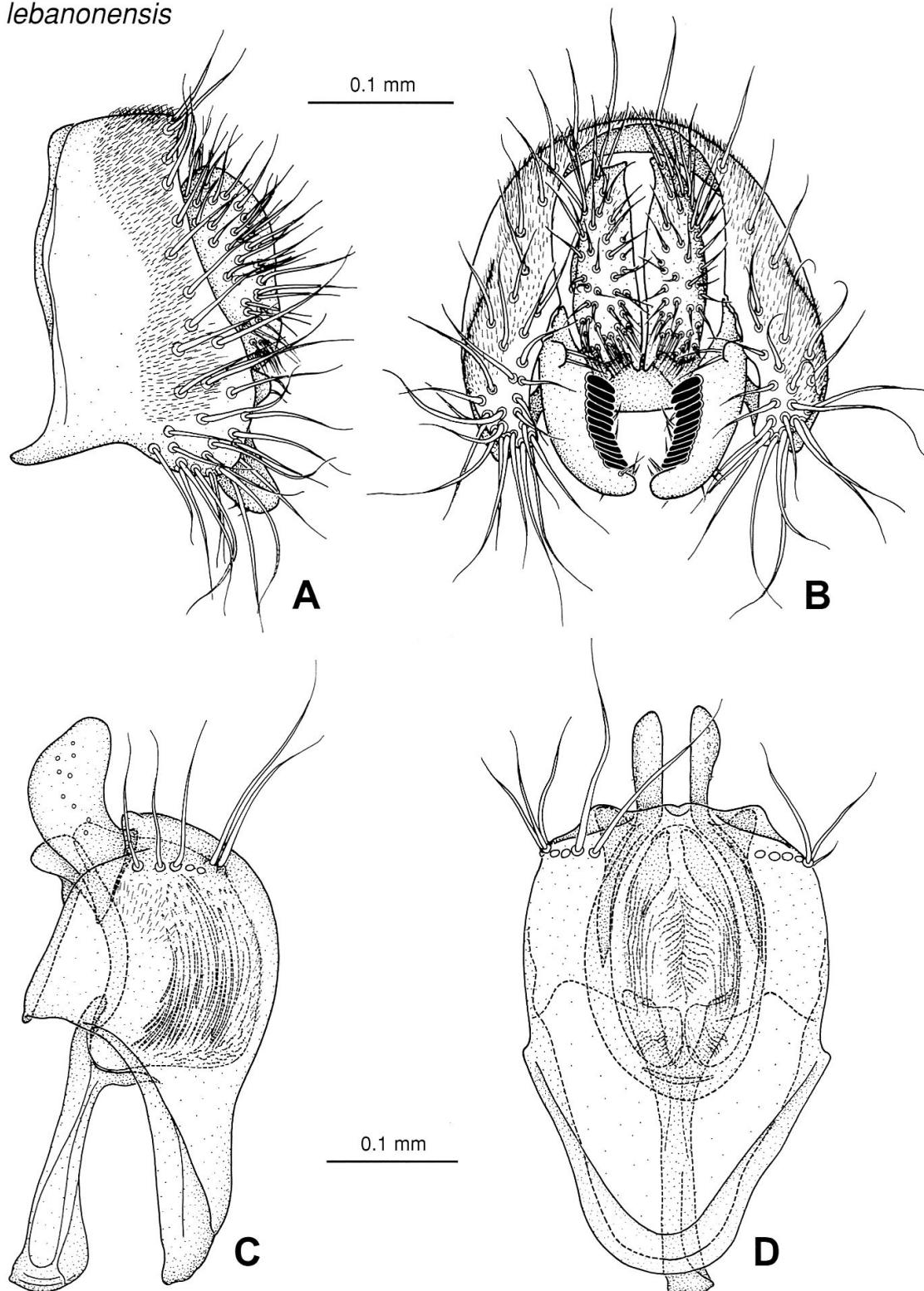
Fig. 3 A–D. *Scaptodrosophila lebanonensis* (Wheeler), specimen from strain 11010–0021.0 (see Tab. 1). A, epandrium, cerci, and surstyli, left lateral view; B, idem, plus decasternum, posterior view; C, hypandrium+gonopods, paraphyses, aedeagus, and aedeagal apodeme, left lateral view; D, idem, ventral view.

Redescription. Measurements based on five pinned males, labelled «E: Gandesa, 31.V.1997 / Strain, L. Serra leg.»

♂. Head. Frons reddish-brown, dull, frontal length 0.32 (0.29–0.38) mm; frontal index = 1.01 (0.95–1.06), top to bottom width ratio = 1.22 (1.19–1.28). Frontal triangle pale brown, subshiny; ocellar triangle prominent, dark brown on inner sides of ocelli, microtrichose, about 35–38 % of frontal length. Interfrontal setulae distinct, arranged in V-shaped rows. Orbital plates with distinctly darker, brownish color, microtrichose, narrow, apically not divergent from eye margin, about 66–75 % of frontal length. Orbital setae black, or2 behind or1, distance of or3 to or1 = 66–72 % of or3 to vtm, or1 / or3 ratio = 0.98 (0.80–1.33), or2 / or1 ratio = 0.33 (0.25–0.42), postocellar setae convergent but not crossed, about 36 (29–39) %, ocellar setae = 73 (65–81) % of frontal length; vibrissal index = 0.56 (0.54–0.60). Face dark brown. Carina pale brown, noselike, bulbous. Cheek index about 5–9. Eye index = 1.32 (1.30–1.33). Occiput brown, with narrow yellowish margin. Antennae pale brown.

Arista with 3–4 rather short dorsal, 2 ventral and about 9 rather long inner branches, plus terminal fork. Proboscis brownish-yellow. Clypeus dark brown. Palpus with about 3 dark and several fine, pale setae along the lower margin.

Thorax length 1.01 (0.81–1.16) mm. Scutum dark brown, shiny, postpronotum yellowish-brown, 6 rows of acrostichal setulae. h index = 1.03 (0.89–1.18). Transverse distance of dorsocentral setae 170–209 % of longitudinal distance; dc index = 0.60 (0.55–0.64). Prescutellar setae elongated, length about 54–71 % of anterior scutellar setae. Scutellum subshiny, distance between apical scutellar setae about 111–144 % of that between apical and basal one; basal ones divergent; scut index = 0.90 (0.79–0.93). Pleura brownish, shiny, sterno index = 0.81 (0.74–0.88), median katepisternal seta about 72–76 % of anterior one. Halter pale yellow. Legs pale brownish, femora distinctly thickened, profemur about twice as wide as antennae, with a row of elongated anteroventral setae which are about as long as 1/4 width of femur; tibiae slightly paler apically, preapical setae on all tibiae, apical seta on mesotibia.

Wing relatively short, apically slightly roundish, hyaline, veins pale brownish, length 2.08 (1.85–2.17) mm, length to width ratio = 2.12 (2.07–2.19). Indices: C = 2.10 (1.75–2.77), ac = 2.67 (2.17–2.83), hb = 0.64 (0.53–0.77), 4C = 1.35 (0.81–1.78), 4v = 2.46 (1.94–3.00), 5x = 2.07 (1.80–2.50), M = 0.78 (0.62–1.00), prox. x = 0.87 (0.62–1.11).

Abdomen dark brown, shiny, some tergites, at least tergites 2–4, slightly yellowish basally.

♂ *Terminalia* (description based on a male with stock number 11010–0021.0, see Tab. 1). Epandrium ventroanteriorly expanded and sharply pointed frontwards, ventrolaterally folded inwards (fold oblique, triangular and apically linked to lateral margin of decasternum by membranous tissue), distally mostly microtrichose, with ca. 23 remarkably long lower setae, and ca. 7 upper setae, which are conspicuously very long and distally waved; ventral lobe medially microtrichose, partially covering surstylus. Cercus narrow, anteriorly connected to epandrium by membranous tissue, dorsally slightly microtrichose, ventral margin folded anterad, devoid of ventral lobe although inner corner of ventral margin is slightly pointed. Surstylus slightly crescentic, not microtrichose, with a concave row of ca. 12 peglike prensisetae, roundish at tip, ca. 10 inner and 2 outer setae, on a small anterodorsal lobe,
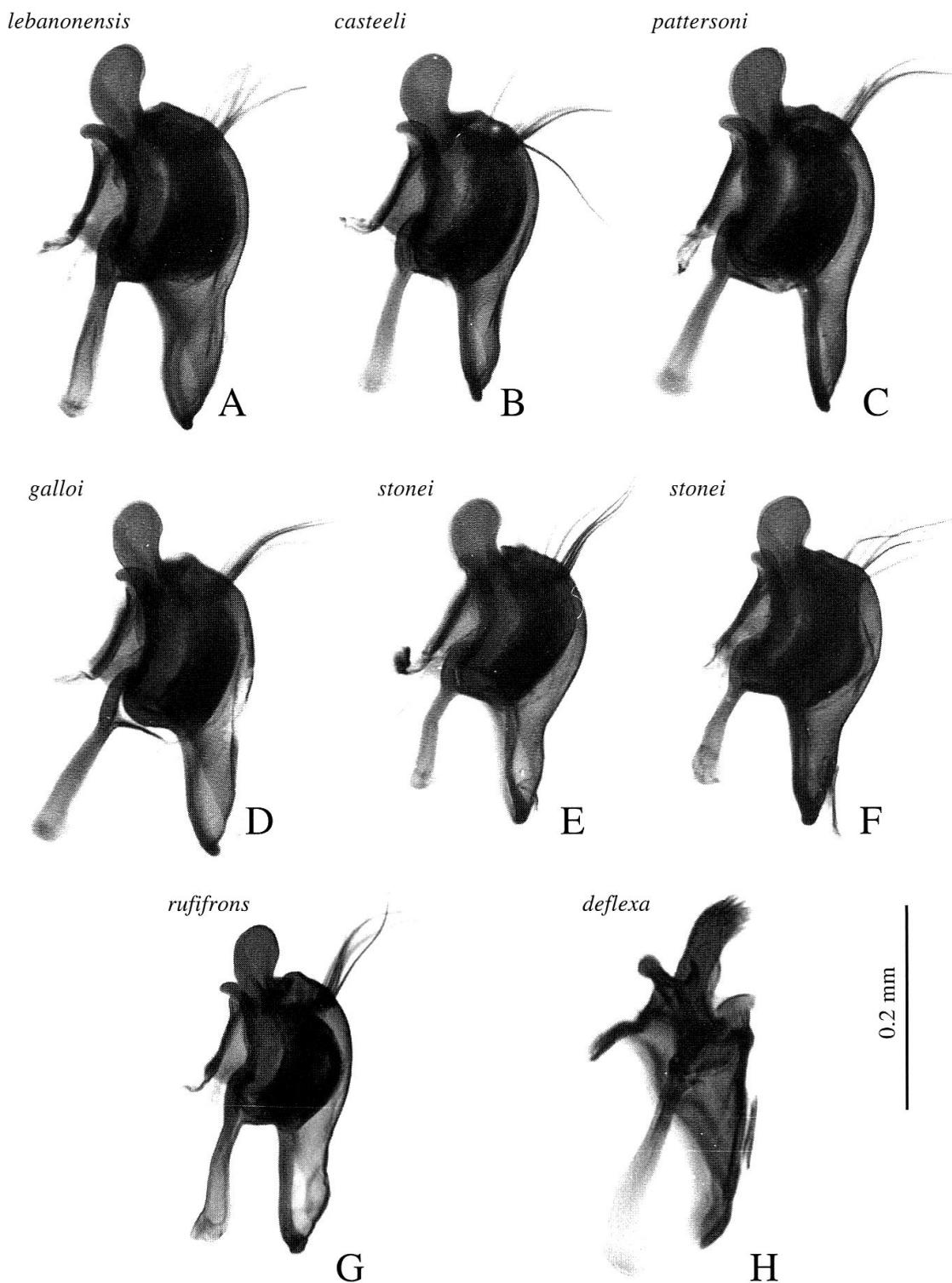
Fig. 4. Photomicrographs of male internal terminalia, left lateral view. Strain numbers are according to Tab. 1: — A, *S. lebanonensis*, strain 11010–0021.0; — B, *S. lebanonensis casteeli*, strain 11010–0011.0; — C, *S. pattersoni*, strain 11010–0031.0; — D, *S. galloi*, strain 11010.0051.0; — E, *S. stonei*, strain 11010–0041.0; — F, idem, strain 11010–0041.1; — G. *S. rufifrons*, Switzerland, labelled «CH: Visp VS, VI–VII.1996 / C. Besuchet leg. / ♂ / *Drosophila rufifrons* L., G. Bächli det.»; — H, *S. deflexa*, Switzerland, labelled «Veyrier GE, X. 1973, H. Beck leg / ♂ / *Drosophila deflexa* D., G. Bächli det.».

just anterior to and above uppermost prensisetae. Decasternum (Fig. 3B) rectangular, laterally membranous, obliquely positioned, distal margin medially notched, where it matches the expanded inner ventral margin of cerci, anterior margin linked by membranous tissue medially to lateral margins of hypandrium, which are anteriorly positioned. Hypandrium longer than epandrium, laterally expanded dorsad, completely embracing aedeagus and laterodistally linked by membranous tissue to apical region of inner paraphysis, anterior margin convex, posterior margin slightly sinuate in ventral view (Fig. 3D); posterior hypandrial process and dorsal arch absent; gonopods completely fused to each other and to hypandrium but recognizable because of their connection to outer paraphyses on laterodistal margin of hypandrium, distally bearing a row of ca. 7 long, distally sinuate setae on each side. Aedeagus hidden by hypandrium, mostly membranous, bag-shaped, globous when inverted, laterally flattened when everted, mostly rugose, each wrinkle ending as a tiny scale, ventrally slighly microtrichose, linked to aedeagal apodeme by membranous tissue, and flanked by two pairs of paraphyses. Inner paraphysis strongly sclerotized, bare, slightly dilated and blunt at tip, laterally linked to aedeagus, distally linked to laterodistal margin of hypandrium, and anteriorly connected to dorsodistal branch of aedeagal apodeme by membranous tissue. Outer paraphysis well developed, upper-positioned, distally boomerang-shaped in lateral view (Fig. 3C), medially bearing an irregular row of ca. 9 setulae, anteriorly connected both to laterodistal margin of aedeagus and to median area of distal, protruded margin of hypandrium («gonopods») by membranous tissue. Aedeagal apodeme longer than aedeagus, laterally flattened, distally bifurcate and curved ventrad. Ventral rod anteroposteriorly flattened, as long as adjacent aedeagal apodeme width and shorter than ventroproximal margin of aedeagus.

Comments. To provide an impression of the small morphological differences between certain *rufifrons* group taxa we are dealing with above, we provide a series of photomicrographs (Fig. 4A–G). In comparison with them, it seems quite clear that *S. deflexa* (Fig. 4H) is not closely related with typical species of the *rufifrons* group.

CONCLUSIONS

Based on the results given above, we want to establish the following taxonomic changes in *Scaptodrosophila*:

— *Drosophila pattersoni* Pipkin, 1956: 251 = *D. lebanonensis* Wheeler, 1949: 143 (**new synonymy**).

— *Drosophila stonei* Pipkin, 1956: 254 = *D. lebanonensis* Wheeler, 1949: 143 (**new synonymy**).

— *Drosophila galloi* Lourenço and Mourão, 1992: 575 = *D. lebanonensis* Wheeler, 1949: 143 (**new synonymy**). *Drosophila dimorpha*, first mentioned in the literature by Mourão *et al.* (1965: 581), is a manuscript name for *S. galloi*, mentioned also in the stock lists of the Tucson *Drosophila* Resource Center; the description of *S. galloi* is based on the same strain, as stated by Lourenço & Mourão (1992: 576).

— *Scaptodrosophila brooksae* (Pipkin, 1961: 152), *Scaptodrosophila ebonata* (Parshad and Duggal, 1966: 279) and *Scaptodrosophila throckmortoni* (Okada, 1973: 436) are removed from the *victoria* group and arranged in the *rufifrons* group.

— The status of *Scaptodrosophila brooksae* and *Scaptodrosophila throckmortoni* remains open, but the first might be an additional synonym of *S. lebanonensis* and the latter a synonym of *S. rufifrons*.

— *Scaptodrosophila deflexa* (Duda, 1924: 222) is removed from the *victoria* group and remains ungrouped.

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Die Verwandtschaftsbeziehungen von fünf Taxa aus den *victoria-* und *rufifrons*-Gruppen wurden mittels DNS-Sequenz-Analysen untersucht. Die Ergebnisse belegen, dass die *rufifrons*-Gruppe monophyletisch ist und zwei Linien enthält, die mit den Arten *S. rufifrons* und *S. lebanonensis* identisch sind. Die Taxa *S. pattersoni*, *S. stonei* und *S. galloi* sind als Synonyme von *S. lebanonensis* anzusehen. Wir schlagen vor, *S. deflexa* aus der *victoria*-Gruppe zu entfernen und die Arten *S. brooksae*, *S. ebonata* and *S. throckmortoni* von der *victoria*-Gruppe in die *rufifrons*-Gruppe zu versetzen. Die *victoria*–Gruppe wird monotypisch und enthält nur noch *S. victoria*, deren Identität unklar bleibt. Eine Neubeschreibung von *S. lebanonensis* ist eingeschlossen.

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