

SHORT NOTE

## An unexpected occurrence of *Amblyseius swirskii* (Athias-Henriot) in La Réunion Island (Acari: Phytoseiidae)

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**ABSTRACT** — The identity of specimens of phytoseiid mites collected inside greenhouses infested by thrips in La Réunion Island was assessed. As the sampled species belongs to the genus *Amblyseius*, presently containing 415 species which are notoriously difficult to discriminate, morphological and molecular traits were evaluated. Both morphological and DNA comparisons showed that the collected specimens belong to the species *Amblyseius swirskii*, a successful biological control agent introduced in the market more than ten years ago in Europe and Northern America. This finding was unexpected, as this species was never reported in this part of the world; an accidental introduction is thus suspected.

**KEYWORDS** — thrips, pepper, roses, biological control agent, 12S rRNA

### INTRODUCTION

Biological control is the most environmentally safe and most economical mode of pest management for growers (Cock *et al.* 2010). Augmentative biological control has been proved to be an efficient alternative to chemical control in vegetable production in greenhouses (van Lenteren and Bueno 2003). The biocontrol industry has made great advances in the last decades with the discovery of more than 230 species of natural enemies available for augmentative biological control worldwide (van Lenteren 2012). Among them, phytoseiid mites are efficient

predators of small insects and mites (McMurtry *et al.* 2013). They are considered important biocontrol agents with many species sold and used all around the world (Wright 2004). The predatory mite *Amblyseius swirskii* Athias-Henriot, 1962 (Acari: Phytoseiidae) is one of the most efficient; it is currently released in more than 50 countries of the world. It originates from the East Mediterranean coast and has been described in 1962 from almond (*Prunus amygdalus* [Miller] D.A. Webb) in Bet Dagan, Israel by Athias-Henriot (1962). This species was then reported along the coast of Israel, Middle Eastern countries, Southern Europe, Sub-Saharan Africa

and the America (Demite et al. 2016).

Three species have been recently established as junior synonyms of *A. swirskii*:

- *Amblyseius capsicum* (Basha, Youssef, Ibrahim & Mostafa, 2001), described and reported from Egypt on *Capsicum annuum* L. (Solanaceae) (Demite et al. 2016).
- *Amblyseius enab* El-Badry 1967, described and reported from Egypt on *Mangifera indica* L. (Anacardiaceae) (Demite et al. 2016).
- *Amblyseius rykei* Pritchard & Baker 1962, described from Congo on *Hoslundia opposita* Vahl (Lamiaceae) and reported in Benin, Cuba, Congo, Ghana, Kenya, Malawi, Nigeria and Zimbabwe (Demite et al. 2016).

Thus, this species is able to develop not only in the Mediterranean basin but also in subtropical and tropical areas (Zannou and Hanna 2011). Since this species is not entering diapause, it can be used throughout much of the season where daytime temperatures regularly exceed 22 °C (Calvo et al. 2015). *Amblyseius swirskii* is commonly used to control whiteflies and thrips in greenhouse vegetables (especially cucumber, pepper and eggplant) and some ornamental crops, in Europe and North America (Calvo et al. 2015). The biology of this species and its importance for biocontrol were recently reviewed by Calvo et al. (2015) and Buitenhuis et al. (2015).

In 2015, a species looking like *A. swirskii* was discovered after a thrips outbreak on peppers and roses at the Réunion Island, at several thousand kilometres from its supposed native area. *Amblyseius swirskii* was furthermore not reported in previous mite biodiversity surveys in La Réunion Island (Quilici et al. 1997, 2000; Kreiter et al. 2002; Kreiter et al. unpub. data). Production of vegetables and ornamentals in greenhouses in this Island correspond to great economic inputs. Furthermore because of recurrent problems with pesticide use (efficiency, environmental and health risks), the development of biological control is very important to improve.

According to the new French regulations on importation of macro-organisms (Anonymous, 2012), *A. swirskii* is currently authorized for sale in the French Metropolitan Area but La Réunion is an

over-seas territory; therefore it is impossible to sell and use this species in this latter area, as it is not indigenous. An importation permit could be requested, but it is expensive and chances to obtain are low. The objective of this paper is thus to assess the identity of the species occurring in the landscape of La Réunion Island in order to determine if the interesting biological control agent *A. swirskii* is occurring in this territory. Furthermore, as the genus *Amblyseius* contains approximately 415 species, as no over-all identification key of species of this genus exists, as many species are morphologically very close (with suspicion of synonymies or cryptic species), both morphological and molecular approaches have been carried to ascertain the diagnosis.

## MATERIALS AND METHODS

### Collection of mites and mountings

The specimens were collected on pepper plants *Capsicum annuum* L. and on roses *Rosa* sp. inside greenhouses in La Réunion Island in two locations: Bassin-Martin (in city of Saint-Pierre territory), Station Armeflhor (lat. 55°31'9" S, long. 21°20'0" E, altitude 450 m) in greenhouse 1 on roses, 13 V 2015 (coll. Olivier Fontaine); Montvert-les-Bas, EARL Le Montvert (lat. 55°32'19" S, long. 21°19'42" E, altitude 582 m), in greenhouse with peppers, 15 V 2015 (coll. Mrs Solène Callarc and Olivier Fontaine); same location, in another greenhouse with peppers, 15 V 2015 (coll. Ingrid Avril) (Table 1). Phytoseiid mites were stored in 95 % ethanol. Part of this material was used for morphological identification; permanent slides were prepared using Hoyer's medium and kept on a hot plate (50 °C) for two weeks. The other part of the material was used for molecular analyses (see below).

### Morphological analyses

The concept of generic classification of Chant & McMurtry (2007) is used in this paper. The terminologies for chaetotaxy are those proposed by Lindquist & Evans (1965) as adapted by Rowell et al. (1978) for dorsal idiosomal setae and by Chant

TABLE 1: Origin and characteristics of studied populations with molecular sequencing and Genbank accession numbers.

Origin	Locality	GPS Coordinates	Altitude	Host plants	Genbank accession numbers
Koppert BV, Commercial strain of <i>Amblyseius swirskii</i>				Rearings	KX064691 KX064692 KX064693 KX064694 KX064695 KX064696
France La Réunion Island Wild strain of <i>Amblyseius</i> sp.	Montvert	55°32'19"S 21°19'42"E	582 m	<i>Capsicum annuum</i>	KX064698 KX064699 KX064700 KX064701 KX064702 KX064703
	Bassin-Martin	55°31'9"S 21°20'0"E	450 m	<i>Rosa</i> sp.	KX064697 KX064704

& Yoshida-Shaul (1991) for ventral idiosomal setae. Adenotaxy and poridotaxy terminologies are those proposed by Athias-Henriot (1975). Measurements were performed using a phase and interference contrast microscope (Leica DLMB, Leica Microsystèmes SAS, Rueil-Malmaison, France) (400x magnification). All measurements are given in micrometers ( $\mu\text{m}$ ). Measurements of specimens collected in La Réunion were compared to measurements of the original description and to measurements of several re-descriptions: specimens from Israel (Porath and Swirski 1965), from several countries in Africa (Zannou *et al.* 2007), Cape Verde (Ueckermann 1992), Egypt (Abo-Shnaf and Moraes 2014), and from Spain (Ferragut *et al.* 2010). All voucher specimens are deposited in the mite collection of Montpellier SupAgro Acarology Collection, UMR CBGP, Montpellier, France.

### Molecular analysis

As individuals identified morphologically were very close to *A. swirskii* with only some very minor morphological differences, as many cryptic species exist among many genera of Phytoseiidae and as it is important to ascertain the identity of the species in the perspective of potential further studies and uses, molecular analyses were conducted to assess the identity of mites collected in La Réunion. Specimens of La Réunion Island were compared to specimens of the commercial strain of *A. swirskii* reared and sold by Koppert BV (Veilingweg 14, 2651 BE

Berkel en Rodenrijs, The Netherlands) (Table 1). DNA was individually extracted from females, using a Qiagen DNeasy tissue kit (Qiagen, Hilden, Germany), according to the DNA extraction protocol described in Kanouh *et al.* (2010). After DNA extraction, females were retrieved from the Qiagen column as described by Tixier *et al.* (2010). A mitochondrial DNA markers (12S rRNA) was used to allow efficient molecular species diagnosis (see e.g. Okassa *et al.*, 2009, 2011; Tixier *et al.* 2011, 2012, 2014). Primers for the amplification of the DNA fragments were as follows: 12S rRNA, 5'-3' TACTATGTTACGACTTAT and 3'-5' AAACTAG-GATTAGATAACC (Jeyaprakash and Hoy 2002).

The PCR reactions were performed in a 25  $\mu\text{L}$  volume, containing 4  $\mu\text{L}$  of mite DNA, 2.5  $\mu\text{L}$  (1mM) of buffer 10X, 1  $\mu\text{L}$  (1.5mM) of MgCl<sub>2</sub>, 0.5  $\mu\text{L}$  (0.05mM for each) DNTPs, 0.175  $\mu\text{L}$  (0.7 mM) of each primer, 0.125  $\mu\text{L}$  (0.625 U) of Taq Qiagen and 16.525  $\mu\text{L}$  of water. Thermal cycling conditions were as follows for the 12S rRNA marker: 95 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, 40 °C for 30 s and 72 °C for 1 min, and final extension phase at 72 °C for 5 min. Electrophoresis was carried out on a 1.5 % agarose gel in 0.5 x TBE buffer during 20 min at 100 V. PCR products were sequenced along both strands with Dynamic ET Terminator Cycle Sequencing kit, and purified ExoSAP-IT (Amersham Biosciences, GE Healthcare Europe GmbH, CS 20529-78457, 24 Avenue de l'Europe, 78457 Velizy-Villacoublay), us-

TABLE 2: Morphological measurements of females and males of various populations of *Amblyseius swirskii* in comparison with the population collected in La Réunion Island (females and males). **In bold:** specimen measurements average  
 VAS: ventrianal shield; MD: movable digit of the chelicera; FD: fixed digit of the chelicera; L1: leg 1; L2: leg 2; L3: leg 3; L4: leg 4;  
 Spermatheca l.: spermathecal length; Shaft: spermatodactyl digit; \*: St1-St5 distances and not St1-St3; <sup>1</sup> in Athias-Henriot (1962); <sup>2</sup> in Ueckermann (1992); <sup>3</sup> in Zannou et al. (2007); <sup>4</sup> in Ferragut et al. (2010); <sup>5</sup> in Abo-Shnaf and Moraes (2014); <sup>6</sup> in Porath and Swirski (1965)

Character	Females						Males			
	Israel 1962*	Cape Verde 1992*	Africa 2007*	Spain 2010*	Egypt 2014*	La Réunion 2016	Israel 1965*	Africa 2007*	Egypt 2014*	La Réunion 2016
<b>Specimens number</b>	-	<b>16</b> specimens	10 specimens	Unknown	8 specimens	<b>10</b> specimens	10 specimens	1 specimen	5 specimens	5 specimens
<b>DS1</b>	-	347-408	338 (326-352)	345-370	375 (358-397)	<b>384</b> (368-400)	255-295	258	<b>275</b> (254-292)	<b>289</b> (280-300)
<b>DS w</b>	-	211-246	<b>205</b> (202-208)	188-202	<b>214</b> (195-224)	<b>233</b> (205-245)	-	190	<b>179</b> (145-203)	<b>180</b> (167-187)
<b>Peritreme extension</b>	j1	j1	j1	j1	j1	j1	?	j1-j3	j1-j3	j1-j3
<b>Solenostomes</b>	-	-	-	7	6	7	7	?	7	7
<b>j1</b>	<b>29</b> (25-32)	31-39	<b>29</b> (24-32)	24-28	<b>30</b> (25-32)	<b>29</b> (25-31)	21-26	<b>25</b>	<b>22</b> (20-24)	<b>24</b> (23-2)
<b>j3</b>	<b>51</b> (46-57)	57-69	<b>53</b> (48-56)	56	<b>55</b> (52-57)	<b>53</b> (50-58)	44-49	<b>43</b>	<b>44</b> (41-47)	<b>44</b> (41-47)
<b>j4</b>	-	8-9	8	9	<b>10</b> (9-15)	8 (8-10)	5-8	7	8 (7-11)	8 (8-10)
<b>j5</b>	-	8-9	8	8	9 (8-10)	8	5-8	7	8 (7-8)	8
<b>j6</b>	-	9-11	9 (8-10)	8	10 (9-11)	8 (8-10)	5-8	8	8 (7-9)	8
<b>J2</b>	8 (7-9)	9-11	9 (8-10)	8	9 (8-10)	8	5-8	7	8 (7-9)	8
<b>J5</b>	-	9-11	7 (6-8)	8	<b>10</b> (8-10)	<b>10</b> (8-10)	5-8	8	8 (7-8)	8
<b>z2</b>	<b>15</b> (13-20)	12-15	<b>12</b> (11-16)	16	<b>14</b> (11-16)	<b>15</b> (13-15)	12-16	<b>13</b>	<b>14</b> (12-16)	<b>15</b> (14-15)
<b>z4</b>	<b>17</b> (15-26)	12-15	<b>13</b> (10-16)	16-22	<b>15</b> (12-18)	<b>15</b> (15-17)	13-18	<b>13</b>	<b>13</b> (10-15)	<b>15</b> (14-15)
<b>z5</b>	-	8-9	7 (6-8)	8-10	8 (7-10)	8 (5-8)	5-8	6	7 (7-8)	8
<b>Z1</b>	-	9-11	9 (8-10)	8-10	<b>11</b> (10-12)	<b>10</b> (8-10)	10-16	8	<b>11</b> (10-13)	8
<b>Z4</b>	<b>72</b> (67-76)	69-77	<b>73</b> (69-78)	76-80	<b>73</b> (70-76)	<b>76</b> (73-80)	49-62	<b>52</b>	<b>52</b> (49-53)	<b>55</b> (55-60)
<b>Z5</b>	<b>108</b> (102-116)	100-126	<b>110</b> (106-115)	110-118	<b>109</b> (105-112)	<b>113</b> (108-114)	73-81	<b>80</b>	<b>74</b> (70-78)	<b>79</b> (78-80)
<b>s4</b>	<b>76</b> (70-81)	74-85	<b>75</b> (70-82)	76-80	<b>78</b> (72-81)	<b>83</b> (78-100)	57-65	<b>59</b>	<b>61</b> (57-64)	<b>63</b> (60-65)
<b>S2</b>	<b>19</b> (17-25)	15-20	<b>13</b> (11-14)	16-22	<b>19</b> (18-21)	<b>18</b> (17-22)	13-18	<b>14</b>	<b>15</b> (13-18)	<b>15</b> (13-18)
<b>S4</b>	<b>12</b> (10-14)	9-11	9 (8-10)	12-14	11 (8-13)	<b>11</b> (10-12)	9-13	9	9 (7-13)	14 (13-18)
<b>S5</b>	-	9-11	7 (6-8)	10-12	<b>11</b> (9-12)	<b>10</b> (10-11)	9-13	9	9 (8-10)	10 (8-11)
<b>r3</b>	-	23	<b>20</b> (19-21)	22-25	<b>25</b> (23-27)	<b>24</b> (23-25)	21-23	<b>20</b>	<b>21</b> (19-23)	<b>21</b> (19-23)
<b>R1</b>	-	12-15	<b>11</b> (10-13)	14-16	<b>15</b> (12-17)	<b>15</b> (13-17)	16-18	<b>14</b>	<b>14</b> (13-14)	<b>13</b> (13-15)
<b>Sge I</b>	<b>33</b> (29-36)	26-31	<b>24</b>	-	<b>29</b> (27-30)	<b>25</b>	-	-	<b>24</b> (21-25)	<b>20</b>
<b>Sge II</b>	<b>32</b> (26-37)	31-39	<b>28</b> (27-29)	-	<b>33</b> (32-35)	<b>33</b> (30-38)	-	<b>25</b>	<b>26</b> (23-28)	<b>29</b> (28-30)
<b>Sge III</b>	<b>37</b> (36-39)	31-39	<b>32</b>	-	<b>36</b> (35-37)	<b>37</b> (33-48)	-	<b>24</b>	<b>26</b> (23-28)	<b>30</b>
<b>Sti III</b>	-	26-28	<b>22</b> (21-24)	-	<b>27</b> (26-28)	<b>26</b> (25-28)	-	<b>20</b>	<b>21</b> (18-23)	<b>28</b>
<b>Sge IV</b>	<b>64</b> (61-66)	57-69	<b>61</b> (56-66)	65	<b>63</b> (60-65)	<b>64</b> (60-67)	39-52	<b>39</b>	<b>45</b> (40-47)	<b>47</b> (45-48)
<b>Sti IV</b>	<b>45</b> (42-47)	46-54	<b>44</b> (40-51)	40-42	<b>47</b> (44-50)	<b>43</b> (40-45)	31-44	<b>35</b>	<b>36</b> (32-38)	<b>38</b> (35-40)
<b>St IV</b>	<b>62</b> (53-68)	57-69	<b>59</b> (53-64)	62-66	<b>66</b> (60-68)	<b>64</b> (63-65)	49-62	<b>50</b>	<b>54</b> (48-58)	<b>54</b> (50-55)
<b>JV5</b>	-	62-77	-	-	<b>72</b> (65-72)	<b>67</b> (63-70)	31-41	-	<b>36</b> (33-39)	<b>29</b> (28-31)
<b>L1 length</b>	-	-	-	-	-	<b>377</b> (362-387)	-	-	-	<b>302</b> (295-315)
<b>L2 length</b>	-	-	-	-	-	<b>289</b> (274-320)	-	-	-	<b>239</b> (225-242)
<b>L3 length</b>	-	-	-	-	-	<b>300</b> (260-332)	-	-	-	<b>252</b> (225-250)
<b>L4 length</b>	-	-	-	-	-	<b>402</b> (382-450)	-	-	-	<b>317</b> (300-317)
<b>St1-St1</b>	<b>61</b> (61-62)	-	-	-	<b>63</b> (55-68)	<b>61</b> (58-65)	-	-	<b>55</b> (52-60)	<b>52</b> (50-53)
<b>St2-St2</b>	-	-	<b>70</b> (69-70)	-	<b>77</b> (71-87)	<b>71</b> (63-78)	-	-	<b>59</b> (56-63)	<b>60</b>
<b>St3-St3</b>	<b>81</b> (78-83)	-	-	-	<b>87</b> (79-91)	<b>85</b> (82-90)	-	-	<b>61</b> (56-66)	<b>60</b> (57-63)
<b>St1-St3</b>	<b>64</b> (61-66)	-	<b>62</b>	-	-	<b>64</b> (63-65)	-	-	-	<b>112*</b> (110-113)
<b>St4-St4</b>	-	-	-	-	<b>91</b> (82-101)	<b>86</b> (80-92)	-	-	<b>47</b> (43-51)	<b>49</b> (48-50)
<b>St5-St5</b>	<b>70</b> (64-76)	-	<b>73</b> (67-78)	-	<b>76</b> (70-81)	<b>68</b> (60-73)	-	-	<b>39</b> (35-43)	<b>39</b> (35-40)
<b>length of VAS</b>	<b>117</b> (106-129)	130	<b>115</b> (110-120)	123-134	<b>132</b> (126-135)	<b>131</b> (125-140)	112-125	<b>113</b>	<b>121</b> (109-128)	<b>110</b> (108-110)
<b>width VAS (ZV2)</b>	<b>83</b> (78-85)	85-90	<b>79</b> (72-86)	89-92	<b>83</b> (71-89)	<b>87</b> (85-90)	-	<b>145</b>	<b>151</b> (123-164)	<b>158</b> (150-160)
<b>width VAS (Anus)</b>	-	-	<b>79</b> (78-80)	-	<b>86</b>	<b>84</b> (83-85)	-	-	-	<b>79</b> (75-80)
<b>Spermatheca l/Shaft</b>	-	-	<b>10</b>	-	<b>11</b>	<b>10</b>	-	<b>19</b>	<b>20</b> (19-21)	<b>21</b> (19-23)
<b>Chel. movable digit</b>	-	-	<b>33</b> (33-35)	-	<b>33</b> (32-34)	<b>33</b>	-	-	<b>25</b> (23-27)	<b>29</b> (25-30)
<b>Chel. fixed digit</b>	-	-	<b>28</b> (28-30)	-	<b>33</b> (31-34)	<b>33</b>	-	-	<b>26</b> (25-27)	<b>31</b> (27-33)
<b>Nb of teeth / MD</b>	-	3	<b>3</b>	3	<b>3</b>	<b>3</b>	<b>1</b>	-	<b>1</b>	<b>1</b>
<b>Nb of teeth / FD</b>	-	11	<b>9-10</b>	11	<b>9-10</b>	<b>9</b>	6	-	6	6

TABLE 3: 12S rRNA mean genetic distances (minimal and maximal values) of *Amblyseius swirskii* specimens collected in La Réunion Island and *Amblyseius swirskii* specimens from rearing units (Koppert)

	<i>Amblyseius swirskii</i> Koppert	<i>Amblyseius swirskii</i> La Réunion
<i>Amblyseius swirskii</i> Koppert	<b>0.014</b> (0.003 - 0.027)	-
<i>Amblyseius swirskii</i> La Réunion	<b>0.017</b> (0.003 - 0.0036)	<b>0.004</b> (0 - 0.010)

ing Megabase 1000 apparatus. The sequences were compared to previously published sequences using BLAST and aligned with CodonCode Aligner (4.0.4.) (CodonCode, Inc., Centerville, MA, USA). The distance matrix was constructed using the Kimura 2-Parameter model using Mega 6.0 (Tamura *et al.* 2013). Genbank Accession Numbers are indicated in Table 1.

## RESULTS

### Morphological analyses

For the females, except for some slightly longer (*s4*) or shorter (*Sge I*) setae and some slightly shorter dimensions of the sternal and genital shields in the La Réunion population, the measurements were very close to all those reported in the original description and further re-descriptions (Table 2). Clearly the differences observed fall into the intraspecific variation as defined in Tixier (2012). Males of the La Réunion population have slightly longer *S4*, *Sge II*, *Sge III* and *Sti III* and shorter *Sge I* and *JV5* (Table 2). Morphological comparisons seem to show that specimens found in La Réunion belong to the species *A. swirskii*.

### Molecular analyses

A fragment of 418 bp was amplified. The mean genetic distance (1.7 %) between specimens collected in La Réunion Island and specimens of *A. swirskii* from Koppert was low. Genetic distances range between 0.3 and 3.6 % (Table 3). Such values are similar to distances observed between specimens of the Koppert population. Furthermore, such distances clearly correspond to intraspecific distances reported for other species of Phytoseiidae (e.g. Okassa *et al.* 2009, 2011; Tixier *et al.* 2011, 2012,

2014). The molecular results thus confirm that the specimens collected in La Réunion Island belong to *A. swirskii*.

### Discussion and conclusion

Molecular and morphological results both allow us to conclude that specimens collected in La Réunion Island belong to the species *A. swirskii*. The origin of the population collected in La Réunion Island remains unknown. The population may originate from previous releases of a commercial strain of *A. swirskii* coming from Europe. *Amblyseius swirskii* has however been reported from another island far away from a continent: Cape Verde, in 1992 (Ueckermann 1992), far before it was sold and used in large numbers in many countries. It can also have come "naturally" or by human commercial activities from Eastern Africa directly or via Madagascar and/or Mauritius.

Several species of thrips and whiteflies occur in vegetable and ornamental crops in greenhouses in La Réunion (Vayssières *et al.* 2001), along with the broad *Polyphagotarsonemus latus* (Banks), and the external demands for alternatives to chemical control are increasing. The potential for development of this indigenous population of *A. swirskii* as a bio-control agent for La Réunion crops, mainly in greenhouses but possibly also in outdoor crops such as citrus orchards (Juan-Blasco *et al.* 2012), is therefore very high.

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