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Brief Inventory of Phytoseiidae (Parasitiformes) Found on Vegetable Crops in the Republic of Congo

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ABSTRACT

According to the last revised catalogue of the mite family Phytoseiidae and the online Phytoseiidae database, only six species of predatory mites have been identified to date in the Republic of Congo (RC). Two species were reported on cassava (*Manihot esculenta*), two on coffee (*Coffea* spp.), one on lemon (*Citrus* spp.), and one on unidentified plants. In this study, we catalogued predatory mites on five plants of economic interest in the RC. Two hundred and forty-seven mite specimens were collected on *Manihot esculenta*, *Solanum lycopersicum*, *Solanum melongena*, *Abelmoschus esculentus*, and *Capsicum* spp. Traditional taxonomy was used to identify the collected specimens. The morphological characteristics of the females were analyzed, including the lengths of the dorsal setae, presence or absence of dorsal and ventrianal setae, shape of insemination apparatus, leg chaetotaxy, and cheliceral dentition. Six species belonging to two sub-families (Phytoseiinae and Amblyseiinae) and five genera (*Amblyseius*, *Euseius*, *Paraphytoseius*, *Phytoseius*, and *Iphiseius*) were identified. Among these six species, only one had previously been observed in the RC; the remaining five species are reported.

1. Introduction

Phytoseiid mites are well known worldwide for their ability to control several pests in vegetable crops. Moreover, the success of biological control programmes greatly depends on the reliability of the specific taxonomic knowledge; indeed, each species has its own bio-ecological

characteristics, including predator-prey relations, which determine their effectiveness in biological control programmes^[1,2]. Therefore, their accurate identification is crucial. In the case of predatory mites, identification is based on the morphological characteristics of the female; specifically, the lengths of the dorsal setae, the presence or absence of the dorsal and ventrianal setae, the shape of

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the insemination apparatus, leg chaetotaxy, and cheliceral dentition [3]. However, this morphological identification system exhibits a major limitation; when only immature stages and/or males are encountered, accurate identification is impossible. In recent years, integrative taxonomy combining morphological and molecular data has enabled many taxonomic questions within the family Phytoseiidae to be solved [4-6]. This approach has made it possible to confirm the status of specimens belonging to the genus *Euseius* originating from the Republic of Congo (RC), which are morphologically close to the species *Euseius fustis* and *Euseius neodossei* [7].

Few studies have evaluated the biodiversity of Phytoseiidae in the RC [8,9] including a single study that reported six species of predatory mites: *Euseius fustis* Pritchard & Backer, *Typhlodromalus aripo* De Leon, and *Typhlodromalus saltus* (Denmark & Matthyse) on *Manihot esculenta* Crantz (Euphorbiaceae), *Euseius neodossei* (Moraes, Ueckermann & Oliveira) and *Euseius baetae* (Meyer & Rodrigues) on *Coffea* spp. and, *Amblyseius sundi* (Pritchard & Backer) on an unidentified plant species [10]. *Typhlodromalus aripo* and *Typhlodromalus saltus* have previously been reported in Cameroon on *Manihot esculenta*. Both *Euseius fustis* and *Amblyseius sundi* have previously been reported in the Democratic Republic of Congo, on *Manihot esculenta* and *Ficus polita* respectively. *Euseius fustis* has also been recorded in Uganda on *Manihot esculenta*. *Euseius neodossei* has been recorded in Kenya on *Cassia* spp. and in Burundi on *Gmelina* spp. [10]. *Euseius baetae*, in Central Africa was only observed in RC. This inventory of the biodiversity of Phytoseiidae mites is incomplete as it was conducted randomly and with the primary objective of studying the pest mites present on *Manihot esculenta*. Other inventories have been conducted in Central Africa, predominantly in the neighboring DRC, where 47 species have been recorded to date [8,11-13], and in Cameroon, where 39 species have currently been recorded [11-13].

In this study, we record the predatory mites identified on five host plants widely consumed by the Congolese population from RC: cassava, tomato, aubergine, okra, and chilli.

2. Materials and Methods

2.1 Acari Survey

Three rounds of sampling were carried out from 2016 to 2019. Mite samples were collected from five host plants namely: *Manihot Esculenta* Crantz 1766 (Euphorbiaceae), *Abelmoschus esculentus* Moench 1794 (Malvaceae), *Capsicum* spp Linnaeus 1753 (Solanaceae), *Solanum melongena* Linnaeus 1753 (Solanaceae) and *Solanum*

lycopersicum Linnaeus 1753 (Solanaceae), in the south of Brazzaville at four sites: MOUNGALI (1), KOMBÉ (2) (Agri-Congo), Groupement Jean Felicien Mahounda (GJFM) (3) (JFM) and Jardin (4) (experimental site) (Figure 1).

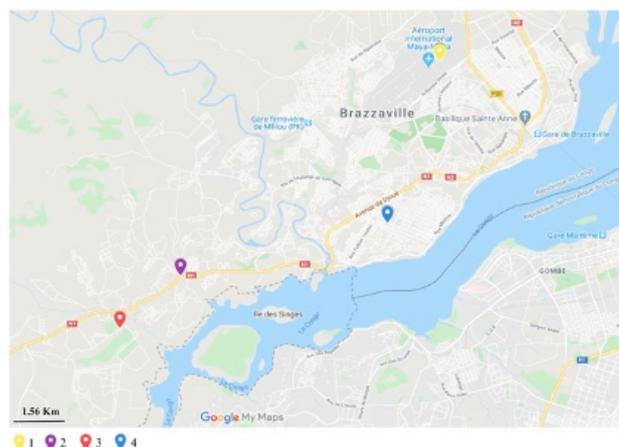


Figure 1. Three rounds of mite sampling from 2016 to 2019

Some females per population (Table 1) were collected directly from the leaves using a fine, clean hairbrush and immediately put in alcohol (70%) in plastic vials. The name of the host plant, sites of collection with GPS coordinates and number of individuals per population were noted on each vial. Males and immature stages were not collected because specific identification is impossible owing to the lack of discriminating characters.

2.2 Morphological Analysis

2.2.1 Traditional Taxonomy

Two hundred and forty-seven adult females were mounted glass slides in Hoyer's medium for later examination under a phase and differential interference contrast microscope (Sony Carl Zeiss Sonnar T* FE 55 mm f/1.8 ZA) at a magnification of 400×. One hundred and four adult females were considered in this study. Terminologies for chaetotaxy used in this paper follow those proposed by Lindquist and Evans (1965) [14] as adapted by H. J. Rowell, D. A. Chant, and R. I. C. Hansell [15] for dorsal idiosomal setae of Phytoseiidae for ventral idiosomal setae. All measurements are presented in micrometres [16]. To differentiate the specimens collected, individuals were measured; the setae considered were those of the sub-tribe of Paraphytoseiina [17], Amblyseiina [18], Euseiina [19], and finally the sub-family Phytoseiinae [3,9]. These specimens were compared with measurements derived from the original descriptions of the following species: *Phytoseius amba* Pritchard and Backer, *Paraphytoseius horrififer* Pritchard and Backer, and *Amblyseius swirskii* Athias-Henriot.

Table 1. Characteristic of different populations of *Amblyseius swirskii*, *Amblyseius* spp., *Iphiseius degenerans*, *Paraphytoseius horriifer*, *Phytoseius amba* and *Euseius fustis*.

Number of females considered	Species	Number of site	Site name	Latitude	Longitude	Host plant
22	<i>Amblyseius swirskii</i>	1	Moungali	4.248112	15.260441	Tomato
1	<i>Amblyseius swirskii</i>	3	Kombé	-4.248112	15.260441	Okra
2	<i>Amblyseius swirskii</i>	1	Moungali	-4.248112	15.260441	Cassava
4	<i>Amblyseius swirskii</i>	1	Moungali	-4.248112	15.260441	Chili
1	<i>Amblyseius swirskii</i>	2	GJFM	-4.310978	15.187236	Cassava
8	<i>Phytoseius amba</i>	2	Kombé	-4.326431	15.170045	Aubergine
1	<i>Phytoseius amba</i>	1	Moungali	-4.248112	15.260441	Okra
30	<i>Euseius fustis</i>	1	Moungali	-4.248112	15.260441	Cassava
10	<i>Euseius fustis</i>	4	Experimental site	4.248112	15.260441	Cassava
13	<i>Euseius fustis</i>	2	GJFM	-4.310978	15.187236	Cassava
15	<i>Euseius fustis</i>	3	Kombé	-4.248112	15.260441	Cassava
12	<i>Iphiseius degenerans</i>	1	Moungali	-4.248112	15.260441	Chili
5	<i>Iphiseius degenerans</i>	1	Moungali	-4.248112	15.260441	Okra
3	<i>Iphiseius degenerans</i>	2	GJFM	-4.310978	15.187236	Cassava
6	<i>Iphiseius degenerans</i>	4	Experimental site	4.248112	15.260441	Cassava
1	<i>Iphiseius degenerans</i>	3	Kombé	-4.248112	15.260441	Aubergine
17	<i>Paraphytoseius horriifer</i>	3	Kombé	4.248112	15.260441	Aubergine
2	<i>Amblyseius</i> spp.	1	Moungali	-4.248112	15.260441	Chili
1	<i>Amblyseius</i> spp.	2	Kombé	-4.248112	15.260441	Aubergine

The morphological characteristics considered were those currently used for the identification of phytoseiid mites [3]; specifically, continuous variables used to distinguish species belonging to the tribu Euseiini [20]. In our study we did not measure individuals belonging to the species *Iphiseius degenerans* due to 1) this species has very small setae and 2) can be identified quite easily. The specimens measured for morphometric analyses were deposited as voucher specimens in the mite collection of the laboratory of Biodiversity and Animal Ecology, Faculty of Science and Technology, Marien Ngouaby University. A donut chart with a hole inside, showing the percentage of each species observed within each host plant studied will be made using the R software and the ggplot2 package [21].

2.2.2 Statistical Approaches

We performed the statistical approach proposed by M-S Tixier [22] to identify continuous variables that can establish boundaries to distinguish between intra- and inter-specific variability based on the lengths of the Phytoseiidae setae on the dorsal shield.

3. Results and Discussion

3.1 Traditional Taxonomy and Statistical Approaches

Six species were identified in this study as belonging to two subfamilies and four genera: Phytoseiinae (*Phytoseius amba*); Amblyseiinae (*Amblyseius swirskii*, *Amblyseius* spp., *Euseius fustis*, *Iphiseius degenerans*, and *Paraphytoseius horriifer*). Cinq of these species have been recorded for the first time in this country.

3.1.1 *Amblyseius swirskii* Athias-Henriot 1962

Examined material/locality: Individuals of this species were found at site 1, 2, and 3.

Distribution: This species was found in proportions of 73.33, 13.33, 10.00 and, 3.33% on *Solanum lycopersicum*, *Capsicum* spp., *Manihot esculenta*, and *Abelmoschus esculentus*, respectively (Figure 2a).

Taxonomic remarks

D. A. Chant and J. A. McMurtry [18] proposed the following stable characteristics for identifying species of the genus *Amblyseius*. A lightly sclerotized idiosoma;

rarely brownish in color; very short z2, z4, Z1, S2, S4, S5, j4, j5, j6, J2, and J5 setae, approximately subequal in length; typically extremely elongate Z4, Z5, and s4 setae. Zannou et al. 2007 identified 23 species of the genus *Amblyseius* native to sub-Saharan Africa^[13]. The continuous characteristics measured on the dorsal shield and the ventrianal shield have the same values as those reported in the original description. However, there is a slight difference in some discontinuous characteristics such as the morphology of Z4 and Z5 setae. The latter is smooth whereas those in the original description are serrated. Moreover, the number of teeth on the mobile digit part varies between six and nine whereas, in the original description, between nine and ten teeth are reported.

3.1.2 *Amblyseius* spp.

Examined material/locality: Individual of this species was found only on site 1.

Distribution: This species was found in proportions of 50 % on *Solanum melongena* and *Capsicum* spp. respectively (Figure 2b).

Taxonomic remarks

Individuals identified as *Amblyseius* spp. typically possess the following characteristics: setae Z1 is absent and setae Z5 and macrosetae on leg IV are shorter than *Amblyseius sundi*. Spermatheca has a tubular calyx and a slightly wider atrium (Figure A1, G × 400). Regarding the chelicera, the movable digit bears three teeth and the fixed digit bears nine teeth (Figure A2, G × 400).

3.1.3 *Paraphytoseius horrififer* Pritchard and Backer 1962

Examined material/locality: Individual of this species was found only on site 3.

Distribution: This species was found in proportions of 100 % on *Solanum melongena* (Figure 2c).

Taxonomic remarks

Three species of the genus *Paraphytoseius* have been reported from sub-Saharan Africa. They have an idiosomal setal pattern 10A: 5D/JV-3/ZV; seta J2, S2, S4, and S5 are absent; one pair of round pre-anal pores posterior is almost in line with JV2; and one pair of metapodal shields. The individuals observed belong to the species *Paraphytoseius horrififer* because they have the following morphological characteristics: dish-shaped calyx of spermatheca, subpentagonal ventrianal shield with an almost straight anterior margin and a lateral margin with

light construction, the peritreme extending to the level of j1, and the last macrosetae absent on leg III. Regarding the chelicera, the movable digit with two-three teeth and the fixed digit with ten-eleven teeth (Figure A3, G×400).

3.1.4 *Euseius fustis* Pritchard and Backer 1962

Examined material/locality: Specimens of this species were found on site 1, 2, 3, and, site 4.

Distribution: This species was found in proportions of 100% on *Manihot esculenta* (Figure 2d).

Taxonomic remarks

Forty-four species belonging to this genus have been reported in sub-Saharan Africa^[11]. Specimens identified as *Euseius fustis* possess the following characteristics: an imbricate, smooth dorsal shield; smooth and serrated Z5 seta; seta Z1 is present the peritreme reaches almost to seta z2; spermatheca with filamentous, trumpet-shaped calyx; and a small and barely distinguishable atrium.

3.1.5 *Iphiseius degenerans* Berlese 1889

Examined material/locality: Individuals of this species were found on the site 1, 2, 3 and 4 **Distribution:** This species was found with a proportion of 44.44, 33.33, 18.51 and 3.70% on *Capsicum* sp., *Abelmoschus esculentus*, *Manihot esculenta* and, *Solanum melongena* (Figure 2e).

Taxonomic remarks

Specimens identified as *Iphiseius degenerans* are characterized by the minute size of all dorsal setae (j3, j4, j5, j6, J2, J5, z2, z4, z5, Z4, Z5, s4, S2, S4, and S5), except for some short vertical setae (j1, Z5). All setae are smooth. The spermatheca has a narrow tubular calyx. Regarding the chelicera, the movable digit bears 1-2 teeth and the fixed digit bears 6-8 teeth.

One species in this genus has been reported in sub-Saharan Africa^[23].

3.1.6 *Phytoseius amba* Pritchard and Backer 1962

Examined material/locality: Individuals were found on the site 1 and 2 .

Distribution: This species was found with a proportion of 88.88 and 11.11 % on *Solanum melongena* and, *Abelmoschus esculentus* (Figure 2f).

Taxonomic remarks

Individuals identified as *Phytoseius amba* typically possess the following characteristics: all setae of species in the genus *Phytoseius* are present (in this genus, setae

Z1, S2, S4, and S5 are typically absent and both setae z3 and s6 are present), except setae Z1, S2, and S5 (Chant and McMurtry 1994); setae j1, j3, z3, Z4, Z5, s4, s6, and r3 are thick, serrated, and long, whereas the other setae are small and smooth; the peritreme extends anterolaterally up to setae j1; and a spermatheca with a slender calyx flares towards the vesicle. Regarding the chelicera, the movable digit bears 1-2 teeth and the fixed digit bears 2-3 teeth. Macrosetae irregularly expanded distally on genu, tibia and basitarsus and knobbed on telotarsus (Figure A4, G × 400). Thirteen species of this genus have been reported in sub-Saharan Africa [24].

3.2 Discussion

Among the six phytoseiid species belonging to two subfamilies and three genera: Amblyseinae (*Amblyseius sundi*, *Euseius neodossei*, *Euseius baetae*, and *Euseius fustis*) and Typhlodrominae (*Thyphlodromalus aripo*, *Thyphlodromalus saltus*) identified in previous study [10] only the *Euseius fustis* species was identified in our study. This has already been reported in the RC, in the Pool and

Bouenza regions and twice at Kombé (1986 and 1987). The absence of species such as *Amblyseius sundi*, *Euseius neodossei*, *Euseius baetae*, and *Euseius saltus* may have been due to the difference in sampling locations and range between the two studies. In this study, we collected specimens only from the south of Brazzaville, whereas (Gutierrez and Bonato 1994) conducted sampling surveys in most regions of the country. All species reported in this paper have been previously described [24,25]. Three individuals of questionable status belonging to the genus *Amblyseius* were also identified. Individuals 12, 13 and 14 were morphologically close to three species: *Amblyseius sundi* Pritchard & Baker and *Amblyseius parasundi* Blommers. According to the Phytoseiidae database [25], two species of the genus *Amblyseius* have been identified in the RC, *Amblyseius genya* Pritchard & Baker and *Amblyseius sundi*. However, the first species has only been observed in the DRC, Cameroon, and Kenya [12,13,26]. Only the *Amblyseius sundi* has previously been reported in the RC. Individuals 12, 13 and 14 sampled in our study differed from this species in the lengths of the Z4, Z5, and s4 setae and the macrosetae of leg IV. Indeed, we

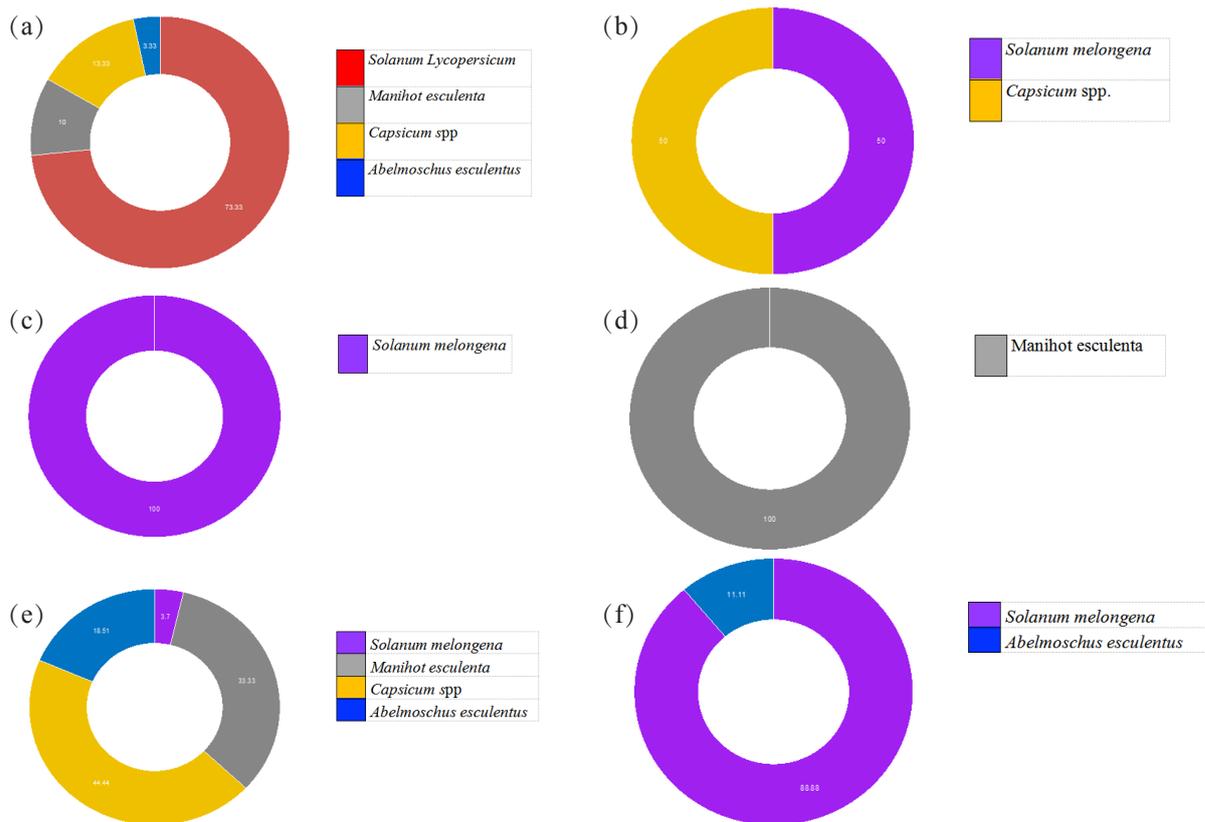


Figure 2. A donut chart with a hole inside, showing the percentage of Phytoseiidae (Parasitiformes) observed within each host plant studied (a) *Amblyseius swirskii*; (b) *Amblyseius* spp.; (c) *Parahytoseius horrififer*; (d) *Euseius fustis*; (e) *Iphiseius degenerans*; (f) *Phytoseius amba*

observed significant differences with the following mean, minimum, and maximum values: 113 (112.5-115), 245 (230-270), 84 (80-87.5), 100 (100-100), 74 (72.5-75), and 62.5 (75-69), whereas the revised description of Zannou et al 2007 [13] gave the following values: 172 (144-208), 445 (336-547), 165 (133-206), 209 (157-270), and 156 (112-208) for Z4, Z5, s4, Sge IV, and Sti IV setae, respectively. Zannou et al. 2007 differentiated *Amblyseius parasundi* from *Amblyseius sundi* based on a Z5 setae and macrosetae on the much shorter leg IV^[13]. However, these specimens were consistently larger than those observed in this study. Indeed, the specimens of *Amblyseius parasundi* had values of 170, 430, and 165 for setae Z4, Z5, and s4, respectively, and values of 190, 140, and 85 for the macrosetae of leg IV, Sge IV, Sti IV, and STIV. Zannou et al 2007 considered that the specimens identified by as *Amblyseius parasundi* were a synonymous species of *Amblyseius sundi*, and that these variations correspond to intraspecific variability. However, when we apply the procedure proposed by M-S Tixier^[22], we observed that the Z4, Z5, and s4 setae and the macrosetae of leg IV can be used to distinguish *Amblyseius sundi* and *Amblyseius parasundi*, and that these variations correspond to inter-specific variability. Indeed, for setae greater than 65 µm, a difference greater than or equal to 31.74 µm is considered to be inter-specific variability.

In view of the criteria listed in Phytoseiid mites of the subtribe Amblyseini (Acari: Phytoseiidae: Amblyseini) from sub-Saharan Africa^[13], we assume that the status of *Amblyseius* spp. raises questions therefore, its status should be validated by further molecular and morphological analyses.

Using integrative taxonomy with the help of molecular markers should help improve the reliability of specific diagnosis in future studies. Indeed, very few DNA sequences from this region exist in the GenBank database^[27]; the only current sequences being those presented in our recent study^[7]. Acquiring knowledge of these parameters is important for the successful implementation of biological control as an alternative pest control method for farmers in the RC. Of the six phytoseiid species recorded in the RC, there is only one species whose life history traits have been thoroughly studied. A controlled laboratory study previously highlighted the potential of *Euseius fustis* in the regulation of cassava green mites (*Mononychellus progresivus* Doreste) when reared using *Mononychellus progresivus* Doreste, as well as pollen from cassava, maize, or castor varieties as food sources^[28]. Future studies should aim to better understand the life history traits of the other 10 phytoseiid species found in the RC to improve the evaluation of their

potential roles in biological control programmes in the inter-tropical zone.

4. Conclusions

Thus, the findings of this study fill some existing gaps in our knowledge in terms of the biodiversity of predatory mites belonging to the family Phytoseiidae in the RC. The number of known mites in the RC is now 10. However, this number is lower than that in the neighboring DRC, where 36 species have been reported. Therefore, it is necessary to conduct further studies to explore the biodiversity of predatory mites in the RC by expanding the study area and number of host plants.

Conflict of Interest

There is no conflict of interest.

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We would like to thank the market gardeners for allowing us to collect the various host plants on which predatory mites live. Without them, this work would not have been possible. We also thank the Pesticides Alternatives association for allowing us to use their phase contrast microscope and camera to identify and capture different individuals collected.

Appendix



Figure A1. *Amblyseius* spp.(spermatheca)



Figure A2. *Amblyseius* spp.(chelicera)



Figure A3. *Paraphytoseius horrifera*



Figure A4. *Phytoseius amba*

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