Article

Sexual morphs and colour variants of *Aphis* (formerly *Toxoptera*) *odinae* (Hemiptera, Aphididae) in Japan

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Abstract

Recent molecular studies have shown that the genus *Toxoptera* is polyphyletic, and in particular that the name of the aphid known since 1952 as *Toxoptera odinae* (van der Goot) should revert to *Aphis odinae* (van der Goot), **stat. rev.** In this paper oviparae, alate males and fundatrices of *A. odinae* are described for the first time, from Japan. *A. odinae* is common as a grey-brown or rust-brown aphid on numerous plant species of shrubby habit throughout the Old World tropics and sub-tropics, but in temperate east Asia much darker forms occur, and Japanese populations of *A. odinae* include a dark green form not found elsewhere in the world. Multivariate morphometrics were used to confirm that both colour forms of the aphid in Japan were conspecific with samples from other parts of the world. *Pergandeida kalopanacis* Hori 1927 is a new synonym of *A. odinae*.

Introduction

Aphis (formerly *Toxoptera*) *odinae* (van der Goot) is a common sight throughout the Old World tropics and sub-tropics on numerous plant species, especially those of shrubby habit. It is known as a mid grey-brown to reddish-brown aphid forming dense colonies on young stems or on the undersides of leaves along the main veins, invari-ably attended by ants. In Africa and most of Asia it reproduces parthenogenetically throughout the year, and until now no sexual morphs have been recorded.

We now report the occurrence of the sexual phase of *A. odinae* on host plants in several different genera in Japan, and describe the ovipara and alate male, and also the fundatrix. Populations in Japan also exhibit a hitherto undescribed colour polymorphism in the parthenogenetic phase, with dark green as well as brown individuals that sometimes occur in mixed colonies, in contrast to the consistently brown colour of this aphid in most countries. We used multivariate morphometrics to demonstrate that the different colour morphs in Japanese populations are all one species, and that they are the same species as found in other parts of the world.

Generic placement of *Aphis odinae*. Van der Goot (1917) originally described this species from Indonesia in the genus *Longiunguis*. It was placed in the genus *Aphis* by Takahashi (1924), but transferred to *Toxoptera* by Eas-top (1952) because of the presence of structures with the potential to function as a stridulatory apparatus; a short row of modified peg-like hairs on the hind tibiae and a ventrolateral series of ridges on the 5th and 6th abdominal sternites. These structures produce an audible sound in the type species of the genus *Toxoptera aurantii* (Boyer de Fonscolombe), in which species the cuticular ridges are particularly well-developed and clearly function as a file or strigil (Broughton & Harris 1971). Other species of *Toxoptera* have peg-like tibial hairs, but the ventrolateral cutic-ular ridges although present are finer and much less evident, and are unlikely to be functional. Weakly developed ventrolateral cuticular ridges are found in many *Aphis* species, including *Aphis nerii* Boyer de Fonscolombe. Rows of peg-like hind tibial hairs are present in certain other Aphidini, including *Aphis eugeniae* van der Goot, and the hind tibiae of the immature stages of many *Aphis* spp. have similar hairs. Peg-like hind tibial hairs are also found in species of three genera of Macrosiphini, although in that tribe a different row of hairs is modified (Holman 1994).

There is thus a strong possibility that the possession of one or other (or both) of these structures is a plesiomorphic character, expressed to a varying extent in different species of Aphidinae.

Recent molecular studies (Kim & Lee 2008, Kim *et al.* 2010, Wang & Qiao 2009) examining both nuclear (EF-1α) and mitochondrial genes, have shown that the genus *Toxoptera* is polyphyletic. In particular, molecular data show that *odinae* does not have a close relationship with other species currently placed in that genus, and clearly belongs within the genus *Aphis*. Accordingly we propose to revert to the combination *Aphis odinae* (van der Goot) **stat. rev.** Two new generic synonymies follow from this; *Arimakia* Matsumura 1917 and *Somaphis* Shinji 1929 were both erected for this species, and therefore become new synonyms of *Aphis* Linnaeus 1758. Further investigation is needed before any firm conclusions can be reached about relationships between the other species currently placed in *Toxoptera*, and about whether they form a cohesive group, either as a separate genus or as a subgenus of *Aphis*.

Descriptions of sexual morphs and fundatrix of *A. odinae*

Oviparae and males of *A. odinae* were collected in the autumn months in several localities in Japan, and on plant genera (*Rhus*, *Viburnum*) in different families. Copulating pairs and oviposition were observed in November on both *Viburnum* and *Rhus*. In addition, specimens collected in 1923 on *Kalopanax pictus* and described by Hori (1927) as *Pergandeida kalopanacis* were re-examined and found to be *A. odinae*. Also, some dark green fundatrices collected on *Juglans* in April 1966 were recognised as this species, together with three dark brown fundatrices collected on *Rhus* in April 2011.

Fundatrix. (Fig. 1a, 2a, 2b) The fundatrix is very dark green or very dark brown, looking bluish black in colour when alive. Its progeny are either bluish black (Fig. 2a) or brown (Fig. 2b). The body is stoutly oval, 2.1–2.4 mm long (excluding cauda), less than 1.5 times longer than wide in mounted specimens. The antennae are relatively short, 5- or 6-segmented, 0.45– 0.53 as long as body excluding cauda; the processus terminalis is 1.54–1.86 times as long as the basal part of the terminal segment; the proportionate lengths of the 3rd−5th segments are 39:23:12+19. The legs are relatively short, with hind tibia 0.42–0.47 as long as body. The hind tibiae have 5-6 very short peg-like setae spaced out along their length. The cuticular ridges on the 5th and 6th abdominal sternites are fine but distinct. The siphunculi are often more conspicuously tapering than in later generations, 0.78–1.56 times as long as their basal widths, with 0–3 setae. The cauda is bluntly tongue-shaped or bluntly conical, 1.05–1.32 times as long as rostral IV+V, and bears 16–24 setae. The genital plate is entire, and bears 31–43 setae.

Specimens examined: Sandankyo Valley, Hiroshima Prefecture, 30-IV-1966, ex *Juglans* sp., M. Miyazaki leg. (Coll. no. MM-1484); Kuratayama, Ise City, Mie Prefecture, 26-IV-2011, ex *Rhus succedanea*, M. Sorin. leg.; Isuzugaoka, Ise City, Mie Prefecture, 29-IV-2011, ex *Rhus succedanea*, M. Sorin. leg.

Ovipara. (Figs 1b, 2c) The body is broadly oval, brown to dark purple-brown in colour when alive, 1.8–2.3 mm in length (excluding cauda) in mounted specimens. The body and appendages bear long fine setae which are 670–830 μm long, 2–3 times as long as the midlength width of the 3rd antennal segment. The antenna are 6-segmented, with 3rd and 4th segments fused together in some cases, 0.54–0.73 as long as body excluding cauda; 3rd segment with 14–22 setae, 4th with 4–18 setae; the processus terminalis is 2.2–3.0 times as long as the basal part of the 6th segment; the proportionate lengths of the 3rd−5th segments are 26:17:21:11+29. The rostrum reaches to the hind coxae, and has segment VI+V 1.28–1.47 times as long as the 2nd segment of hind tarsus, bearing 2, or rarely 3, accessory setae.

The legs have tibiae pale with dark apices in fore and middle legs, infuscated throughout with darker apex in hind leg; the hind tibiae are swollen, bearing many scent plaques which vary in number from about 10 to 200; the peg-like setae on the hind tibiae are distinct, 3–9 (mostly 5–8) in number; the first tarsal chaetotaxy is typically 3:3:2, but fore and middle legs very often devoid of the sense peg resulting in 2:2:2, 2:3:2 or 3:2:2.

The abdomen has a pair of marginal tubercles consistently on the 2nd segment well as those on the 1st and 7th segments, sometimes also on the 3rd; those on the 7th segment are 15–35 μm on longest axis and often elongate to finger-shaped. The abdominal terga are weakly areolated in anterior segments; the 6th tergite often has sclerites at bases of setae in spinal area, which are more developed on the 7th tergite and form a sclerotic band on the 8th; the 8th tergite has 11 – 18 setae. The ventral pattern of "stridulatory" ridges is fine but distinct. The siphunculi are roughly imbricated, cylindrical to tapering, 1.2–2.0 times as long as their basal widths, 0.48–0.62 as long as the cauda, and

often bear 1 or 2 setae. The cauda is bluntly tongue-shaped, usually with a weak constriction at about midlength, 0.76–1.34 times as long as rostral IV+V, with 16–25 setae. The genital plate is vertically divided in the middle, and bears 32–50 setae.

Specimens examined: Sapporo, Hokkaido, 22-X-1923, ex *Kalopanax pictus*, M. Hori leg.; Tsukuba, Ibaraki Prefecture, 2-XI-2009 (Coll. no. MM-09050) and 15-XII-2010 (Coll. no. MM-10012), ex *Viburnum erosum*, M. Miyazaki leg.; Kuratayama, Ise, Mie Prefecture, 19-XI-2010 (Coll. no. MM-10007) & 1-XII-2010 (Coll. no. MM-10013) , ex *Rhus succedanea*, M. Sorin leg.

Male. (Figs 1c, 2c) The male is alate, with media of forewing twice-branched. The body is 1.48–1.75 mm long (excluding cauda) in mounted specimens. The abdomen is blackish brown with irregular shading of dark green when alive, with a narrow dark band on 8th segment, some small sclerites on 6th–7th segments, and small ante- and postsiphuncular sclerites.
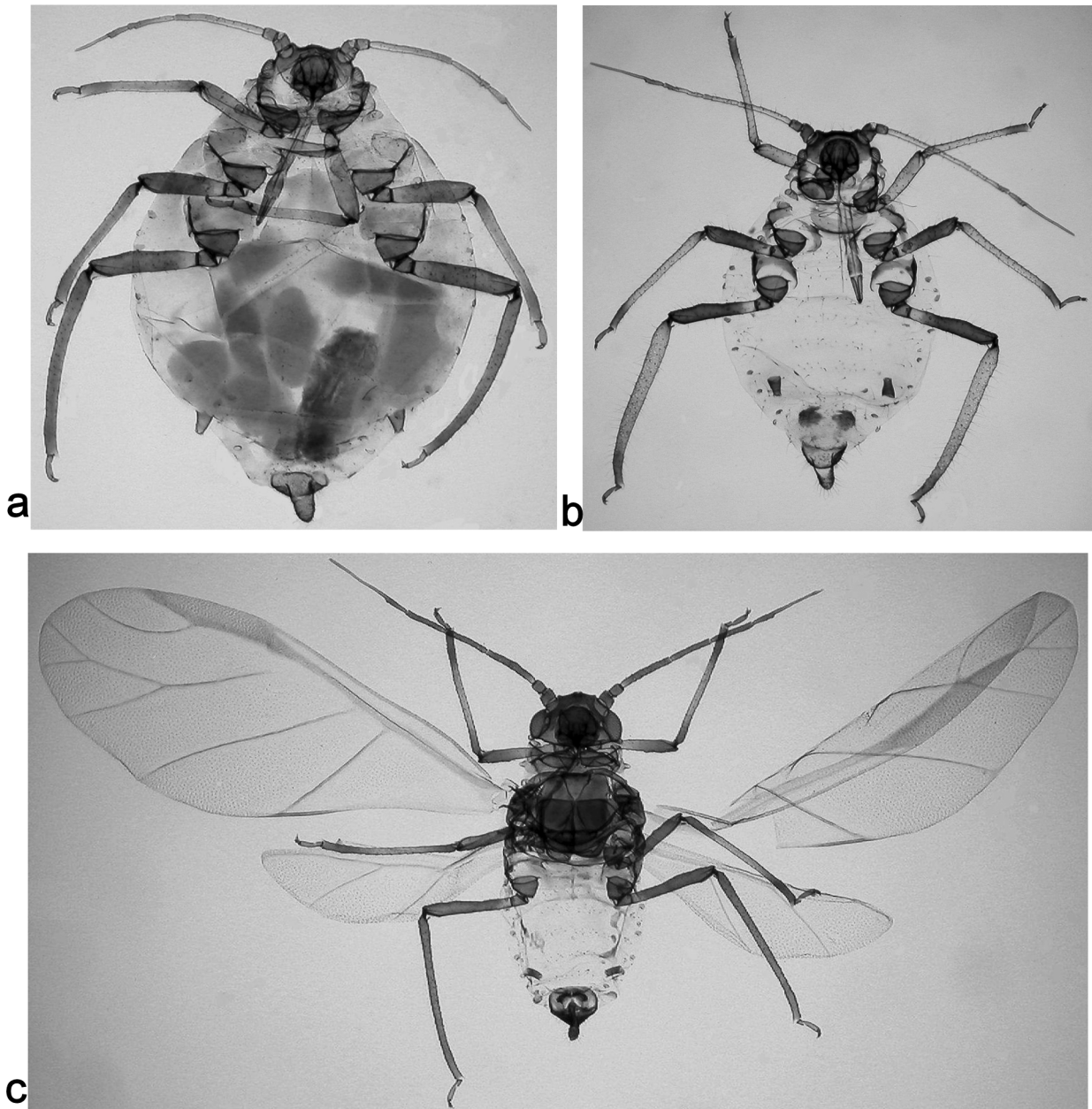

FIGURE 1. Slide-mounted specimens of *A. odinae*: **a,** fundatrix; **b,** oviparous female; **c,** male.
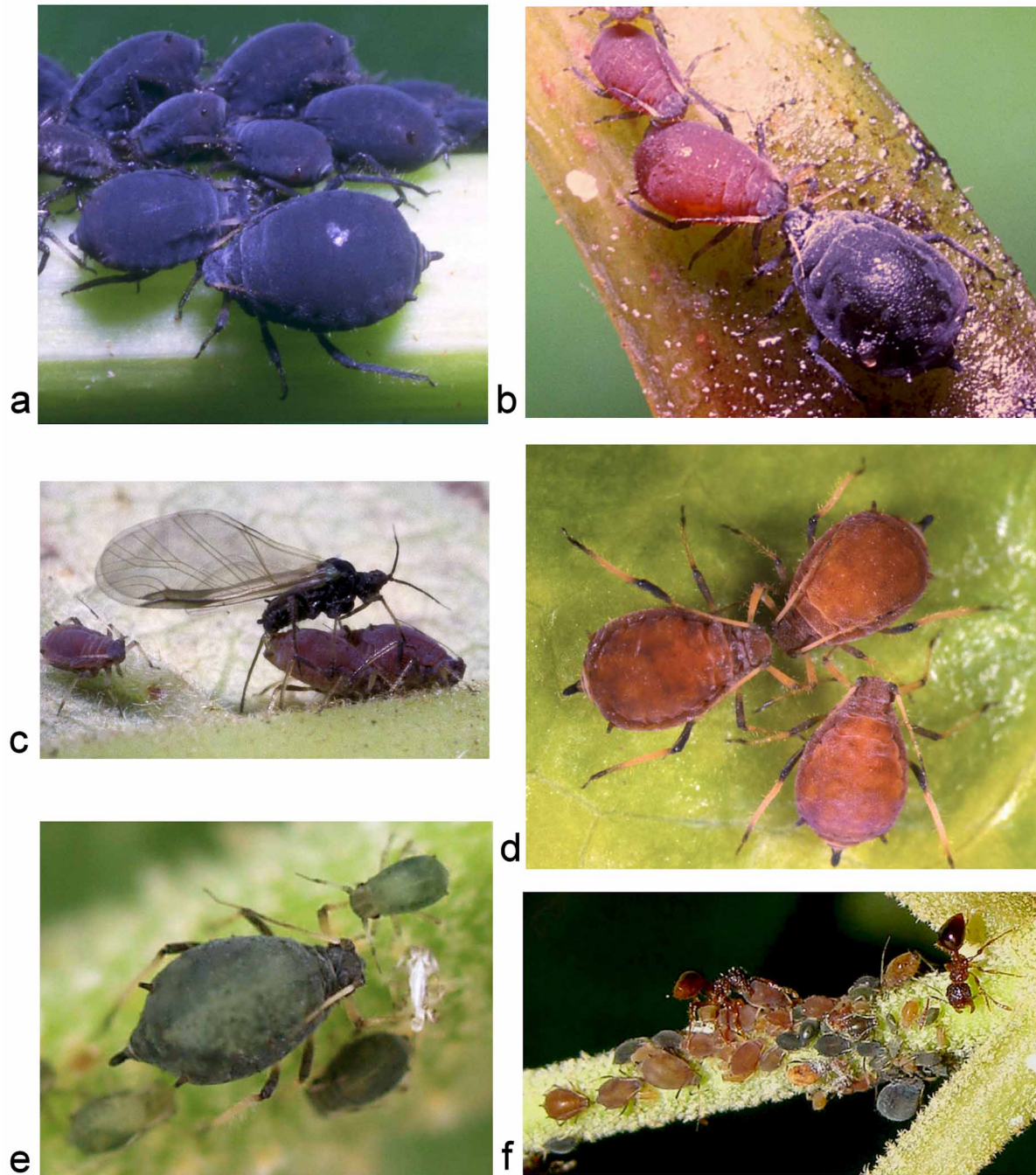
FIGURE 2. Colour photographs of living *A. odinae*: **a,** fundatrix with bluish black progeny; **b,** fundatrix with brown progeny; **c,** oviparae and male; **d,** apterous viviparae of brown form (photograph by courtesy of Dr Poorani Janakiraman); **e,** apterous vivipara of green form; **f,** colony with mixture of the two colour forms.

The antennae are 6-segmented, wholly fuscous, 0.64–0.80 times as long as body; 3rd segment with 13–32 secondary rhinaria, 4th with 5–25, 5th with 4–16 secondary rhinaria; 6th with processus terminalis 2.61–3.35 times as long as basal part of the segment; the proportionate lengths of the 3rd–5th segments are 26:20:20:11+32. The hind tibiae each have a row of 6-9 short peg-like hairs.

The abdomen has marginal tubercles on the 1st and 7th segments; those on the 7th segment are well developed, 28–43 μm on longest axis, hemispherical to conical in shape. There are a series of interconnected finely spiculose ridges on the 5th and 6th abdominal sternites. The siphunculi are cylindrical, nearly parallel-sided or slightly broadened at base, 1.35–2.78 times as long as wide at base. The 8th abdominal tergite bears 2–4 setae. The cauda is bluntly conical in shape, 0.71–0.86 as long as rostral IV+V, and bears 12–14 setae.

Specimens examined: Tsukuba, Ibaraki Prefecture, 2-XI-2009, ex *Viburnum erosum*, M. Miyazaki leg. (Coll. no. MM-09050; Kuratayama, Ise, Mie Prefecture, 18-XI-2010 (Coll. no. MM-10005), 19-XI-2010 (Coll. no. MM-10007) and 20-X-2010 (Coll. no. MM-10008), ex *Rhus succedanea*, M. Sorin leg.

Colour variation in Japanese *A. odinae*. Populations of *A. odinae* in most parts of the world are consistently grey-brown to reddish brown in life (Fig. 2d), but in temperate east Asia populations are often of a much darker brown colour (see, for example, Lee *et al*. 2002). In 2009 a dark green form with apterous viviparae apparently identical in morphology to the brown form was collected at Tsukuba, Ibaraki Prefecture, on *Viburnum erosum* and *Aralia alata* (Fig.2e). Some colonies had a mixture of the two colour forms (Fig. 2f). Twenty apterae of each colour form were reared on excised *Viburnum* leaves. The green mothers produced exclusively green progeny, and the brown mothers produced exclusively brown progeny, showing that colour was stably inherited from one partheno-genetic generation to the next.

Morphometric studies. The morphometric study was based on 208 specimens of adult apterous viviparae from 23 samples of *A. odinae*, 11 from Japan and 12 from other countries (Table 1). The Japanese samples included two samples of the dark green form from Tsukuba. Each sample was collected on a single plant at one location on the same date. Twelve measurements were taken from each specimen: body length excluding cauda (BL), total antenna length (ANT), lengths of antennal segments ANT III, ANT IV, ANT V, ANT VI base (VIb) and processus terminalis (PT), length of combined rostral segments IV and V (R IV+V), length of second segment of hind tarsus (HT II), lengths of siphunculus and cauda (SIPH and CAUDA), and length of longest hair on ANT III (LH III). Ten of these were included as variables in the multivariate analysis; total antennal length was excluded as it was highly correlated with III-PT, and hair length on III was also excluded because the hair ends were too fine to be measured with sufficient accuracy.

TABLE 1. Samples of apterous viviparous females of *Aphis odinae* used in morphometric analysis.

| Sample no.	Locality, host, date and slide identification	Colour	No. of specimens
1	JAPAN: ex *Viburnum* 5.vii.09 Miyazaki 09024A	green	12
2	JAPAN: ex *Viburnum* 5.vii.09 Miyazaki 09024B	brown	11
3	JAPAN: ex *Aralia alata* 10.vii.09 Miyazaki 09025A	brown	11
4	JAPAN: ex *Viburnum* 24.vii.09 Miyazaki 09032	brown	12
5	JAPAN: ex *Viburnum* 21.ix.09 Miyazaki 09035	brown	10
6	JAPAN: ex *Viburnum* 21.ix.09 Miyazaki 09036	brown	12
7	JAPAN: ex *Viburnum* 21.ix.09 Miyazaki 09037	green	10
8	JAPAN: ex *Kalopanax* 28.vi.09 Miyazaki 09038/9	brown	9
9	JAPAN: ex *Rhus succedanea* 10.vii.09 Miyazaki 09045	brown	11
10	JAPAN: ex *Rhus javanica* 26.vii.09 Miyazaki 09047	brown	11
11	JAPAN ex *Rhus javanica* 28.vi.09 Miyazaki 09014	brown	7
12	HONG KONG: ex *Rhus hypoleuca* ii.1982 144/82	brown (?)	6
13	HONG KONG: ex *Sapium* sp. 24.xi.1999 JHM 7276A	brown (?)	8
14	HONG KONG: *Ailanthus fordii* 25.xi.05 JHM 8186	brown (?)	7
15	JAVA: *Stephania forsteri* 14.v. 1948 (Rappard)	brown (?)	12
16	JAVA: *Lannea coromandelica* 16.iv.1950 (Rappard)	brown (?)	9
17	INDIA (Assam): *Mangifera indica* iv.1965 (CIBC)	brown (?)	8
18	INDIA (Orissa): cashewnut 6.v.1957	brown (?)	8
19	SINGAPORE: ?Murraya 19.i.1990 JHM 5601	brown	9
20	PHILIPPINES: *Mussaenda philippica* 5.viii.1965	brown (?)	6
21	INDIA: 'Arabica' coffee i.1995 CIE A16894	brown (?)	9
22	KENYA: *Pittosporum* 17.viii.1993 VFE 19541	brown	6
23	KENYA: banana leaves 15.vi.1988 JHM 5306	brown	5
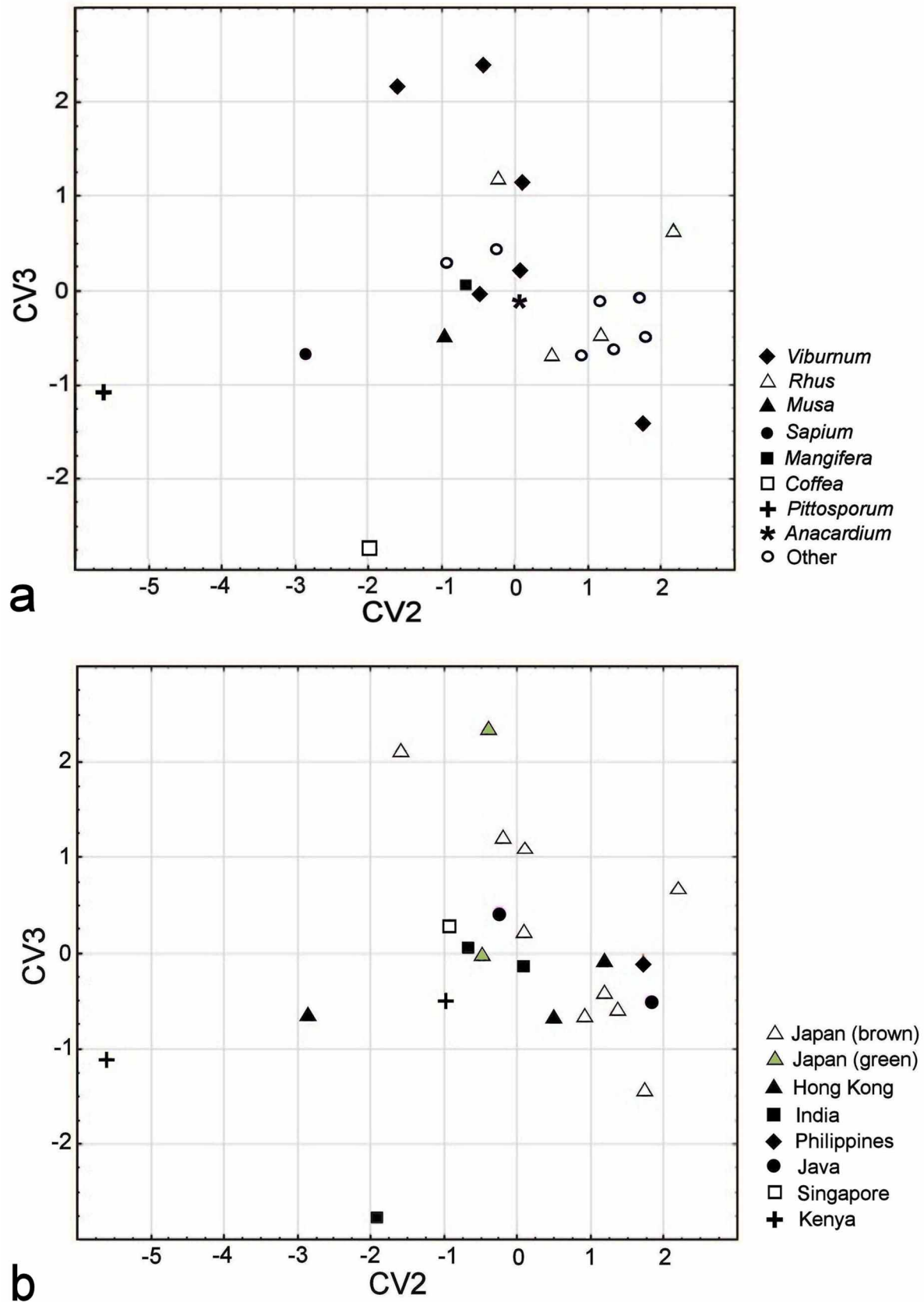
FIGURE 3. Plot of the mean scores on the first two canonical variates (CVs) of 23 samples of *A. odinae*: **a,** labelled according to the host plants on which they were collected; **b,** labelled according to their countries of origin and colour.

All variables across all samples were normally distributed with no significant departures from normality. Ratios that might discriminate between closely related species (PT/VIb, R IV+V/HT II, SIPH/CAUDA) also had normal distributions across all samples, suggesting that there might only be a single taxon. There was no significant difference in overall variance between samples from localities in Japan where populations were undergoing sexual reproduction, and those from other countries where reproduction is presumed to be permanently parthenogenetic; that is, no effect of possible differences in reproductive mode on variance could be detected.

Multiple discriminant (canonical variates) analysis was performed using the 23 samples as the grouping variable. This method maximises the differences between groups (= samples), and has proved to be powerful in separating the environmental and genetic components of variance (Blackman & Spence 1994) and thus resolving differences between closely-related species (Blackman 1992, Blackman & De Boise 2002). Analysing the data for all 23 groups, the first five canonical variates (CVs) accounted for 89.3% of the total variance in the data, of which CV1 accounted for 46.2%, CV2 for 22.4%, CV3 for 11.3%, CV4 for 5.8% and CV5 for only 3.6%. CV1 was strongly correlated with ANT III ($r^2 = 0.83$), which is a reliable measure of general size, whereas none of the other CVs showed any significant size correlation. Thus almost half the total variance in the sample can be accounted for by size differences between samples, which are largely determined by the environment. A large contribution of environment to total variance is consistent with the hypothesis that all the samples belong to one taxon. Having removed the major source of environmental variation, a plot of CV2 against CV3, accounting for 80% of the remaining variance, might be expected to reveal any differences of genetic or taxonomic significance. There was no clustering of the sample means of scores on CV2 or CV3 according to host (fig. 3a), geographical location or colour (fig. 3b), providing a strong indication that all samples were conspecific.

Discussion

A. odinae was previously among a number of well-known and widely distributed aphid species considered to be permanently parthenogenetic, with no known sexual morphs. It now seems probable that this species is native to Japan or that part of temperate east Asia where there is an annual sexual generation, and that one or more permanently parthenogenetic lineages have spread through the tropics and subtropics, where they are either inhibited from producing, or have lost the ability to produce, sexual morphs. This situation is similar to that of *Toxoptera citricidus* (Kirkaldy) and *Aphis nerii*, both also widely distributed in the warmer parts of the world and believed to be permanently parthenogenetic, until sexual morphs were found in Japan, (Komazaki 1988, Takada & Miyazaki 1993). An ovipara of *Toxoptera aurantii* was recently reported from Malta, and alate males of that species have been trapped in Israel (Pérez Hidalgo & Mifsud 2011), but it is not known whether *T. aurantii* has a functional bisexual generation somewhere in the world.

A. odinae is a very polyphagous aphid in its parthenogenetic phase, tending to colonise plants of a certain growth habit rather than to select particular plant species, and there is no indication that it is any less polyphagous in the hosts that it utilises for sexual reproduction. In this respect it differs from many other species, especially those that host-alternate, which may be very selective in their choice of host for the sexual phase. In such species the lack of a sexual phase in introduced populations could be attributable to the absence of a suitable host for this part of the life cycle, but in *A. odinae* this is unlikely to be a limiting factor.

Some species of Aphidini vary greatly in colour depending on the environmental conditions under which they develop. Apterae of *Aphis gossypii* Glover, for example, vary in colour from dark blackish green or green mottled with dark green (larger specimens in favourable conditions) to very pale whitish yellow (small specimens in crowded colonies or hot conditions). However, the colour variation in apterae of *A. odinae* in Japan clearly has a genetic basis, and this is unusual in Aphidini, although genetically-determined colour polymorphism occurs frequently in the sister tribe Macrosiphini. There are also considerable differences in colour between the different morphs in the life cycle of *A. odinae*, which may be one reason why the sexual morphs and fundatrices have not previously been recognised as this species, and in particular may have led Hori (1926) to describe the dark-coloured form as another species (*Pergandeida kalopanacis*).

Acknowledgements

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