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First Report and Distribution of the Indian Mustard Aphid, *Lipaphis erysimi pseudobrassicae* (Hemiptera: Aphididae) on Cabbage (*Brassica oleracea* var *capitata*) in Ghana

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Abstract

The presence of large colonies of aphids is associated with a devastating novel necrotic disease of cabbage (*Brassica oleracea* var. *capitata*) in Ghana that is thought to be of viral etiology. In this study, we used molecular taxonomic tools to identify the aphid species present on these diseased cabbage plants. This was confirmed using two key features for morphological identification, involving the length of cornicles and shape of cauda for the wingless forms of the aphids. Two species of aphids were identified and their distribution in Ghana indicated. One was the generalist aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) but the most abundant was the brassica specialist aphid, *Lipaphis erysimi pseudobrassicae* (Davis) (Hemiptera: Aphididae), which is one of the most destructive pests of brassica crops in some countries in Africa and other parts of the world. *L. erysimi* has been reported in Benin, Mali, South Africa, India, China, and United States, but this is the first formal report of *L. erysimi pseudobrassicae* in Ghana. The correct identification of *L. erysimi* is crucial, suggesting that it has recently become one of the most common species of aphid found on cabbage plants in Ghana.

Key words: first report, identification, aphids, virus, cabbage

Aphids represent a major constraint to the production of many crops worldwide, especially brassica crops (mustards and crucifers) (Hughes 1963, Yue and Liu 2000, Opfer and McGrath 2013, Fening et al. 2016). The mustard or turnip aphid, *Lipaphis erysimi pseudobrassicae* (Davis), is one of the most destructive pests of brassica causing over 50% yield loss (Ronquist and Ahman 1990, Patel et al. 2004, Carter and Sorensen 2013, Adhab and Schoelz 2015). These aphids feed by sucking sap from their host plants, leading to stunted growth, and they excrete honeydew, which leads to fungal growth (sooty mold) (Hughes 1963, Blackman and Eastop 1984). Furthermore, aphids of the *L. erysimi* group transmit over 13 different viruses, including important viruses of the Brassicaceae, such as *Turnip mosaic virus* (*potyvirus*), *Beet mosaic virus* (*Potyvirus*), *Cauliflower mosaic virus Caulimovirus*, and *Radish mosaic virus Comovirus* (Kennedy et al. 1962, Wang et al. 1998, Adhab and Schoelz 2015). The turnip aphid, *L. e. pseudobrassicae* is a serious pest of cruciferous crops that is native in Asia where it has a wide distribution (Tran et al. 2016). In addition, the aphid has apparently been introduced into many other countries outside Asia (Essig 1948). The turnip aphid was earlier confused with the cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae), due to its close resemblance, and became well established before it was recognized as a distinct species (Essig 1948). Moreover, its true identity was not discovered until 1914 when it was described as *Aphis pseudobrassicae* by Davis (1914, p. 231) (Essig 1948).

The turnip aphid can seriously damage the crops by removing photoassimilates and by transmitting at least 16 plant viruses, and is often difficult to manage (Chan et al. 1991, Tran et al. 2016). Significant damage caused by this aphid on crucifers has been reported in many African countries including Benin, Mali, South Africa, Sudan, and Egypt (Daiber 1971, Müller 1986, James et al. 2010, Saethre et al. 2011).

The correct identification of aphids is essential in agriculture because of the differences between species, biotypes, and strains

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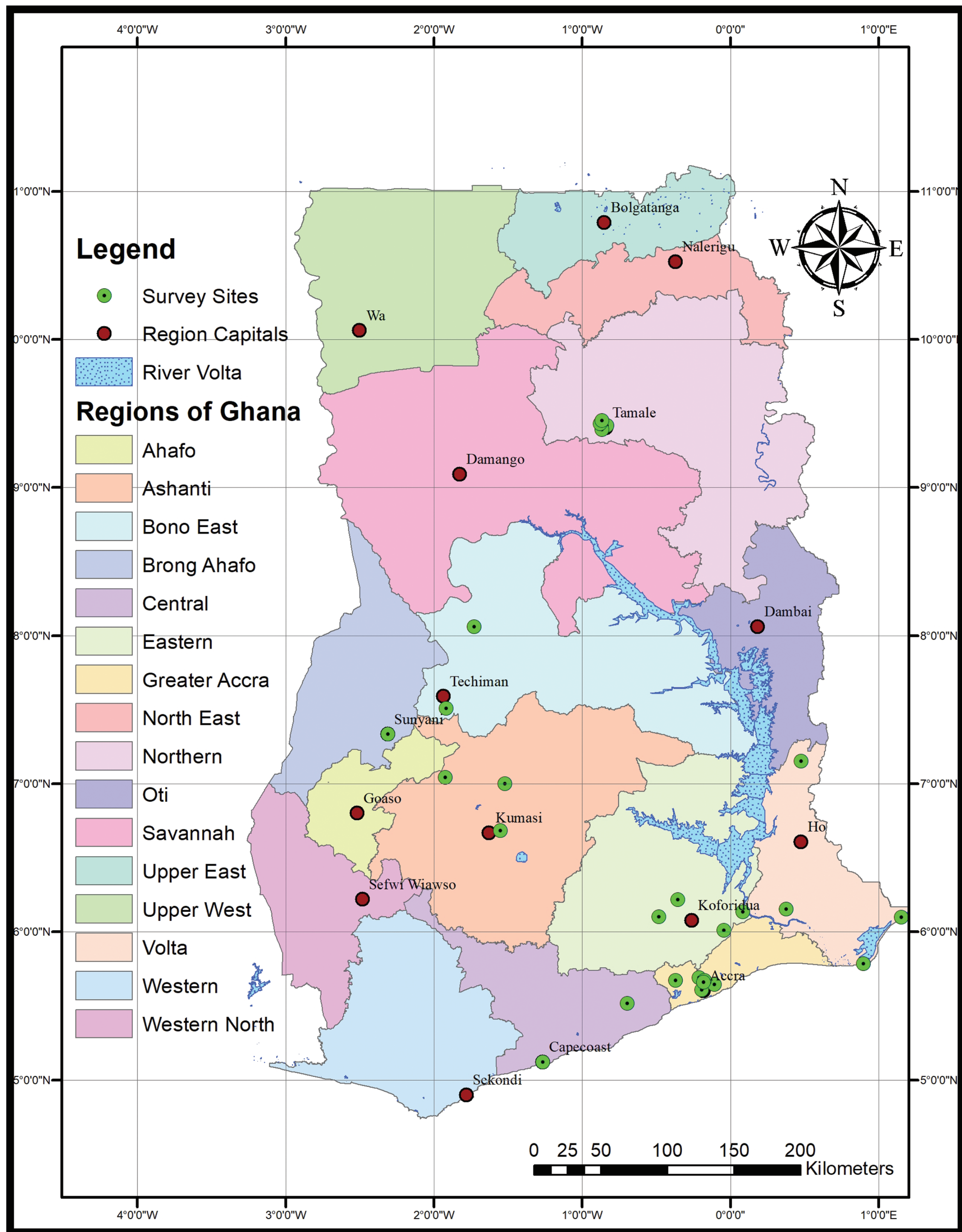

Fig. 1. Map of Ghana showing surveyed locations.

in insecticide susceptibility and the transmission of viruses (Eastop 1977, Nauen and Elbert 2003). This gives appropriate insights into which control strategy will be the most appropriate. *L. erysimi* occurs in both the tropics and temperate regions and is still sometimes confused with the cabbage aphid (Yue and Liu 2000, Capinera 2008). The cornicles of both of these aphid species are relatively short, but the body of *B. brassicae* is covered with a clearly visible grayish waxy covering, which contrasts with a thin layer of white,

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Table 1. Surveyed sites for presence of *L. e. pseudobrassicae* with geographic coordinates

Region	Agro-ecological zone	Community	Cabbage variety	Latitude	Longitude
Greater Accra	Coastal Savanna	Dzorwulu	Oxylus	5.6096245	−0.1939582
		Tema	Oxylus	5.6464175	−0.1101984
		Agbogba	Oxylus	5.69054106	−0.212493704
		Madina	Supercross	5.67150077	−0.177876186
		Legon Univ. farm	Oxylus	5.66163	−0.18346
Eastern	Coastal Savanna and semi-deciduous forest	Kpong	Oxylus/supercross	6.134874	0.08207
		Aseseeso	Oxylus/Santa	6.009533	−0.0443213
		Tafo	Oxylus	6.216210	−0.357507
		Densuso	Oxylus	6.10267822	−0.485384182
		Kyekyere	Oxylus	5.67150077	−0.372393113
Northern	Guinea Savanna	Sangani	Oxulus	9.41728021	−0.834687485
		Sawaba	kkcross	9.39140386	−0.867386616
		Kpalesi	Oxylus	9.43068524	−0.879671495
		Gurugu	Santa	9.45323849	−0.86767002
Ashanti	Semi-deciduous forest	KNUST	Oxylus	6.684633	−1.554009
		Akanfo	Oxylus	7.00093	−1.52166
		Opokukrom	Oxylus	7.04252	-1.92509
Central	Coastal Savanna and semi-deciduous forest	Cape Coast	Oxylus	5.121389	−1.268333
		Agona Swedru	Oxylus	5.518402	−0.69881
Volta	Coastal Savanna and semi-deciduous forest	Anloga	Oxylus/Santa	5.784232	0.894835
		Denu Lowcost	Oxylus	6.098252	1.150434
		Hohoe	Oxylus	7.15185	0.47383
		Sogakope	Oxylus/kkcross	6.152568	0.374835
Bono East	Semi-deciduous forest	Sunyani	Oxylus	7.33494	−2.3123
Brong Ahafo	Transitional	Techiman	Oxylus	7.51027	−1.91852

Table 2. Key morphological features used to identify *Lipaphis erysimi pseudobrassicae* and *Myzus persicae*

Feature	*Lipaphis erysimi pseudobrassicae*	*Myzus persicae*
Cornicles	Cornicles are not dark and distinctly longer than the cauda	Cornicles are the same color as the body and are long, >2 times the length of the cauda
Cauda	Tongue-shaped	Cone-shaped

Source: Blackman and Eastop (1984).

waxy secretions on *L. erysimi* (Carter and Sorensen 2013, Opfer and McGrath 2013).

Two morphologically similar but clearly distinct geographically separated forms or subspecies of mustard aphid are known to occur; namely the European form, *Lipaphis erysimi erysimi*, and *Lipaphis erysimi pseudobrassicae* which constitutes the Indian strain (Müller 1986, Ronquist and Ahman 1990). European strains of the mustard aphid are known to be darker in color and seemingly prefer other host plants (mainly *Sisymbrium officinale*) than the strains from India and elsewhere. For these reasons, the European strain of mustard aphid has been referred to as *L. e. erysimi* as distinguished from *L. e. pseudobrassicae* (Müller 1986). Until this classification, *L. e. pseudobrassicae* was referred to as *L. pseudobrassicae* (Davis) and *L. e. erysimi* was referred to as *L. erysimi* (Kaltenbach) (Müller 1986). *L. e. pseudobrassicae* is known as the false cabbage aphid due to its close resemblance to the cabbage aphid, *B. brassicae* (Müller 1986, Ronquist and Ahman 1990).

Therefore, the distribution and economic impact of these two brassica specialists are sometimes incorrectly reported. Until this study, *L. e. pseudobrassicae* had not been formally reported in Ghana and there was no available data on its distribution.

Materials and Methods

Sampling and Identification of Aphids

Individual adult aphids, both winged and wingless, were collected using a fine brush from cabbage (*Brassica oleracea* var. *capitata*) plants in Kpong, Aseseeso, and Kumasi, Ghana in August 2015. Some individuals were stored in 96% ethanol at 4°C for subsequent morphological examination, and other individuals were stored in *RNAlater* (Qiagen, Hilden, Germany) at −20°C for molecular genetic analysis. The morphological examination was carried out with a Leica EZ4 D stereomicroscope with 40× magnification and specimens were identified using the keys of Blackman and Eastop (1984).

Molecular identification of the aphids was done by sequencing of the *Cytochrome c oxidase subunit I* (*CO I*) gene. Total genomic DNA was extracted from individual aphids. Each aphid was picked using forceps from the tubes where they had been preserved in 95% (v/v) ethanol and the ethanol blotted off on a tissue paper pad. The aphid was macerated in 150µl of 'TRIZol-like' reagent [made up of 38% v/v Tris-buffered phenol, 0.8 M guanidine thiocyanate, 0.1 M sodium acetate pH 5.0, and 5% (v/v) glycerol] in a microfuge tube using a micropestle. In total, 15 µl of 24:1 chloroform: isoamyl alcohol was added and the mixture vortexed and placed on ice for 5 min. The mix was then centrifuged at 10,000 × *g* for 5 min and the aqueous layer transferred to a sterile microfuge tube. An equal amount of isopropanol prechilled to −20°C was added and the mix kept at −20°C for at least 2 h. To pellet the nucleic acids, the mixture was centrifuged at 10,000 × *g* for 10 min at 4°C in a prechilled desktop centrifuge and the isopropanol poured off. The pellets were washed once by mixing with 70% (v/v) ethanol then centrifuged at 10,000 × *g* for 5 min. The ethanol was carefully drained off and the pellet air-dried to remove all traces of ethanol before being dissolved in 25 µl sterile water. The DNA was quantified using a Nanodrop spectrophotometer and the DNA diluted to 50 ng/µl for use in the PCR reaction.

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PCR was carried out using Biomix Red premix (Bioline Reagents Ltd.) according to the manufacturer's instructions. Briefly, 10 µl of the premix was mixed with 8 µl of nuclease-free water and 1 µl of the cDNA template (50 ng/µl) added. The universal primer pair HCO 2198 5′-TAA ACT TCA GGG TGC CAA AAA ATCA-3′ and LCO 1490 5′-GGT CAA CAA ATC ATA AAG ATA TTGG-3′ were used to amplify an approximately 700 base pair DNA fragment of the mitochondrial gene (Folmer et al. 1994). PCR cycling conditions were initial denaturation 94°C, 3 min followed by 30 cycles at 94°C for 30 s, an annealing step at 55°C, an extension step at 72°C for 1 min and a final extension of 72°C degrees for 10 min on a thermal cycler (ABI-Applied Biosystems, Veriti).

Successful PCR amplification was confirmed on a 1.5% agarose gel. PCR products were purified by selective adsorption to silica gel membranes under controlled ionic conditions using the MinElute PCR purification kit (Qiagen, Hilden, United Kingdom) according to the manufacturers' instructions. The purified PCR products were quantified using a Nanodrop spectrophotometer (Thermo Scientific) and diluted to the required concentration specified by the sequencing service provider (Source Biosciences Lifesciences, Nottingham, United Kingdom). All the amplicons were sequenced by automated Sanger sequencing (Sanger et al. 1977, Smith et al. 1986) in both the forward and reverse directions using the respective forward and reverse primers used to generate them. Initial sequence identification was done by checking for homology using BLAST (Altschul et al. 1990). Pairwise comparison was done to establish closeness and identity of the two aphid species from Kpong, Aseseeso, and Kumasi in Ghana to those from elsewhere in the gene bank. The sequences generated through Sanger sequencing for *L. pseudobrassicae* (synonymous to *L. e. pseudobrassicae*) and *M. persicae* and some comparator sequences from GenBank were aligned using the MUSCLE algorithm and the percentage similarity computed using SDT software (Muhire et al. 2014).

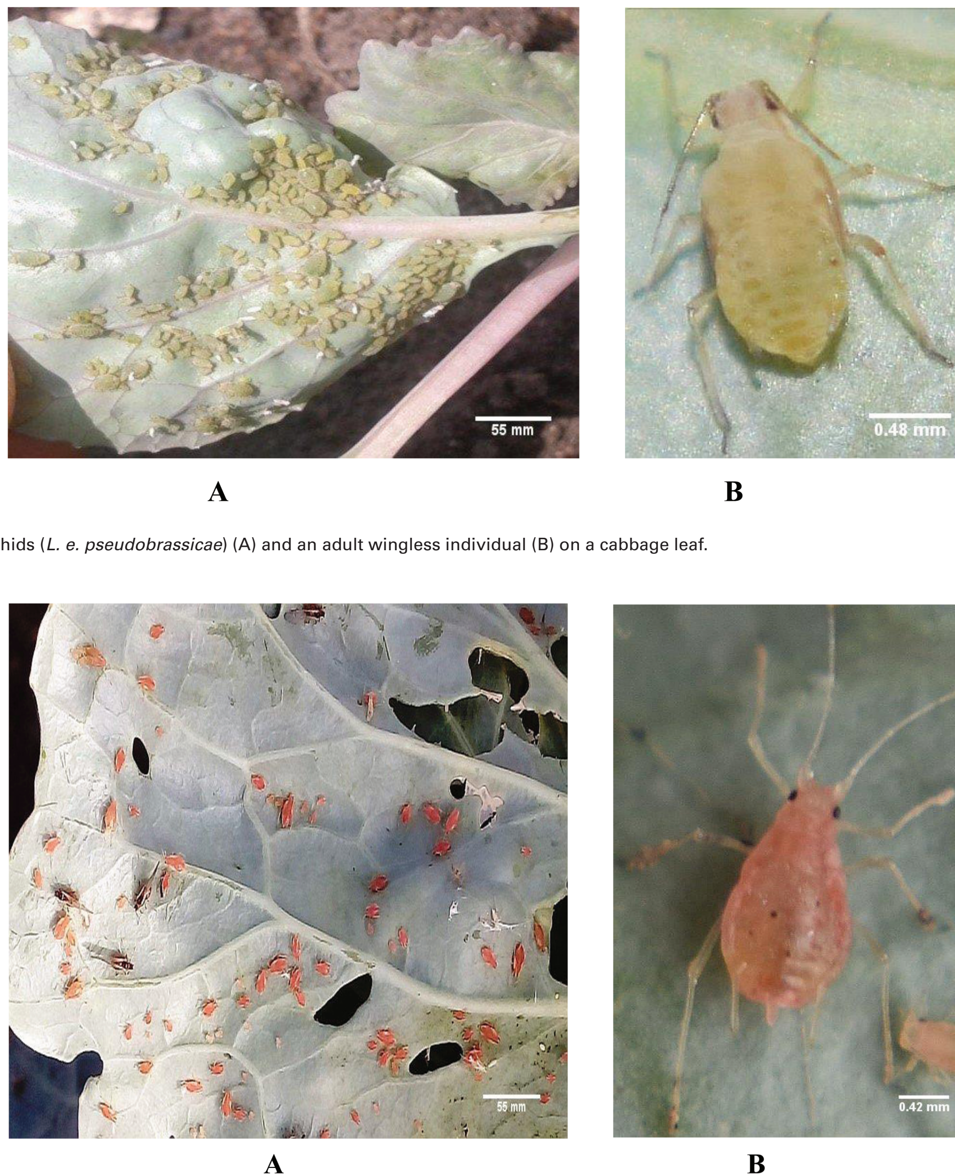

Fig. 2. Colony of aphids (*L. e. pseudobrassicae*) (A) and an adult wingless individual (B) on a cabbage leaf.

Fig. 3. Colony of aphids (*M. persicae*) (A) and an adult wingless individual (B) on a cabbage leaf.

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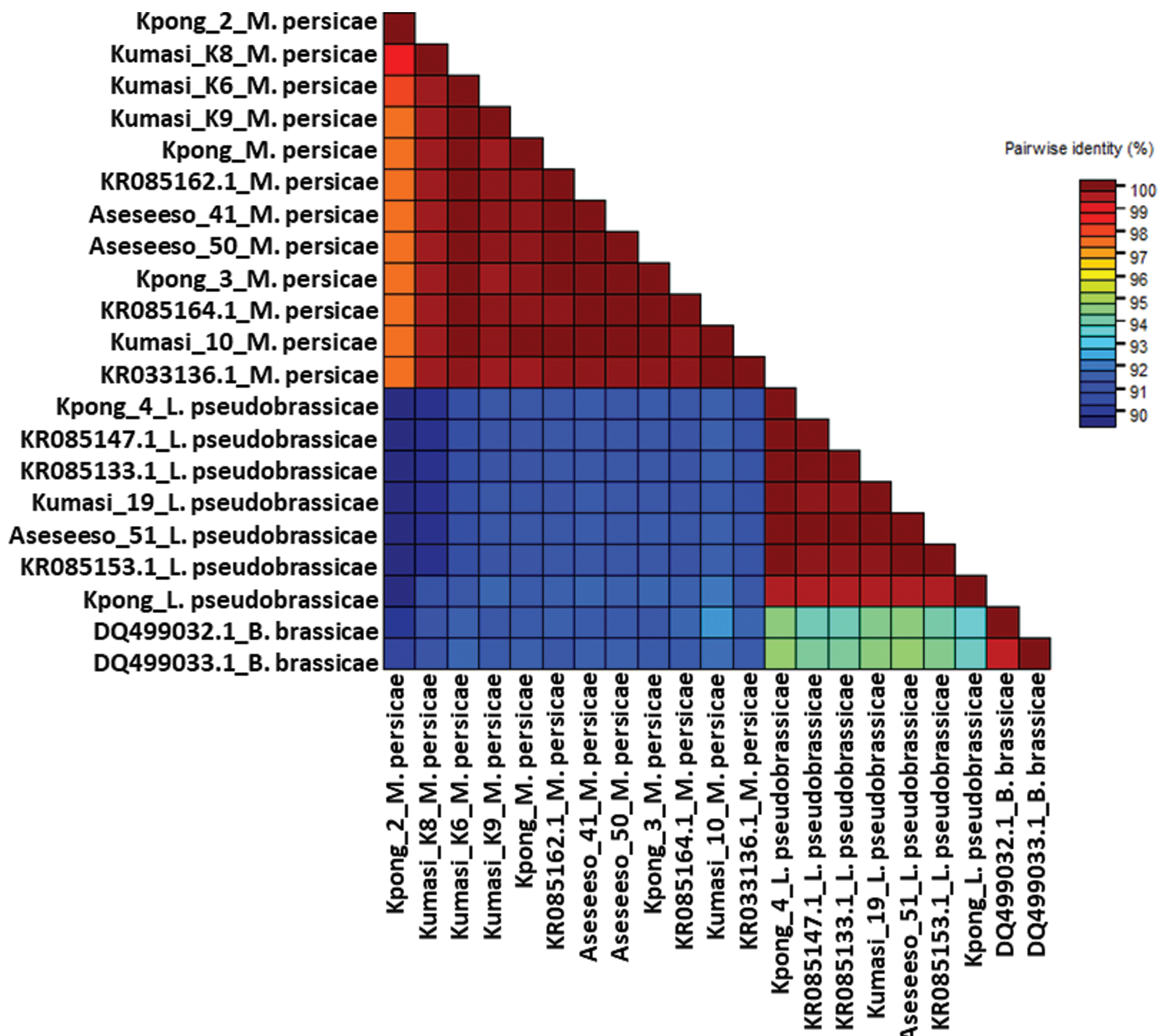

Fig. 4. Pairwise comparison of *CO1* gene sequences. Sequences generated through Sanger sequencing for *Lipaphis pseudobrassicae* (synonymous *L. e. pseudobrassicae*) and *Myzus persicae* and some comparator sequences from GenBank were aligned using the MUSCLE algorithm and the percentage similarity computed using SDT software (Muhire et al. 2014). Closest similarities were within species with percentage differences showing clear species demarcation.

Field Sites for Distribution Studies of *L. e. pseudobrassicae* in Ghana

After *L. e. pseudobrassicae* was identified in 2016, further studies were carried across the major cabbage growing regions in Ghana, belonging to four agro-ecological zones, to establish its distribution. The survey was conducted in 2019 during the major and minor rainy seasons from May to October in 8 regions (Fig. 1), namely Central, Eastern, Greater-Accra, Northern, Volta, Ashanti, Bono East, and Brong-Ahafo, which are the major cabbage producing areas (MoFA 2019). In total, 26 sites were surveyed for the presence of *L. e. pseudobrassicae*. The surveyed locations with their geo-referenced positions (recorded using a GPS Coordinates app with a signal accuracy of 3–5 m) are shown in Table 1. The range and average annual rainfall per each agro-ecological zone is indicated below. The semideciduous rainforest is 1,200–1,600 mm (1,500 mm), Transitional zone is 1,100–1,400 mm (1,300 mm), Coastal Savanna is 600–1,200 mm (800 mm), and Guinea Savanna is 800–1,200 (1,000 mm). Generally, the major rainy season receives a greater amount of the rainfall, and spans from March to July, with the minor season receiving less rainfall, that can be erratic, from September to November, depending upon the agro-ecological zone (Meteorological Department, Legon, Accra, Ghana).

Sampling Procedure for Survey of Aphids

In each region, cabbage farms in different districts were selected for the survey. During the study period, trips were made to the selected sites when cabbages were at least 2 wk old. Twenty (20) plants were randomly sampled per farm, two leaves per plant, following a 'Z' pattern. The aphids found on the leaves were collected using a fine brush into vials containing 70% alcohol for

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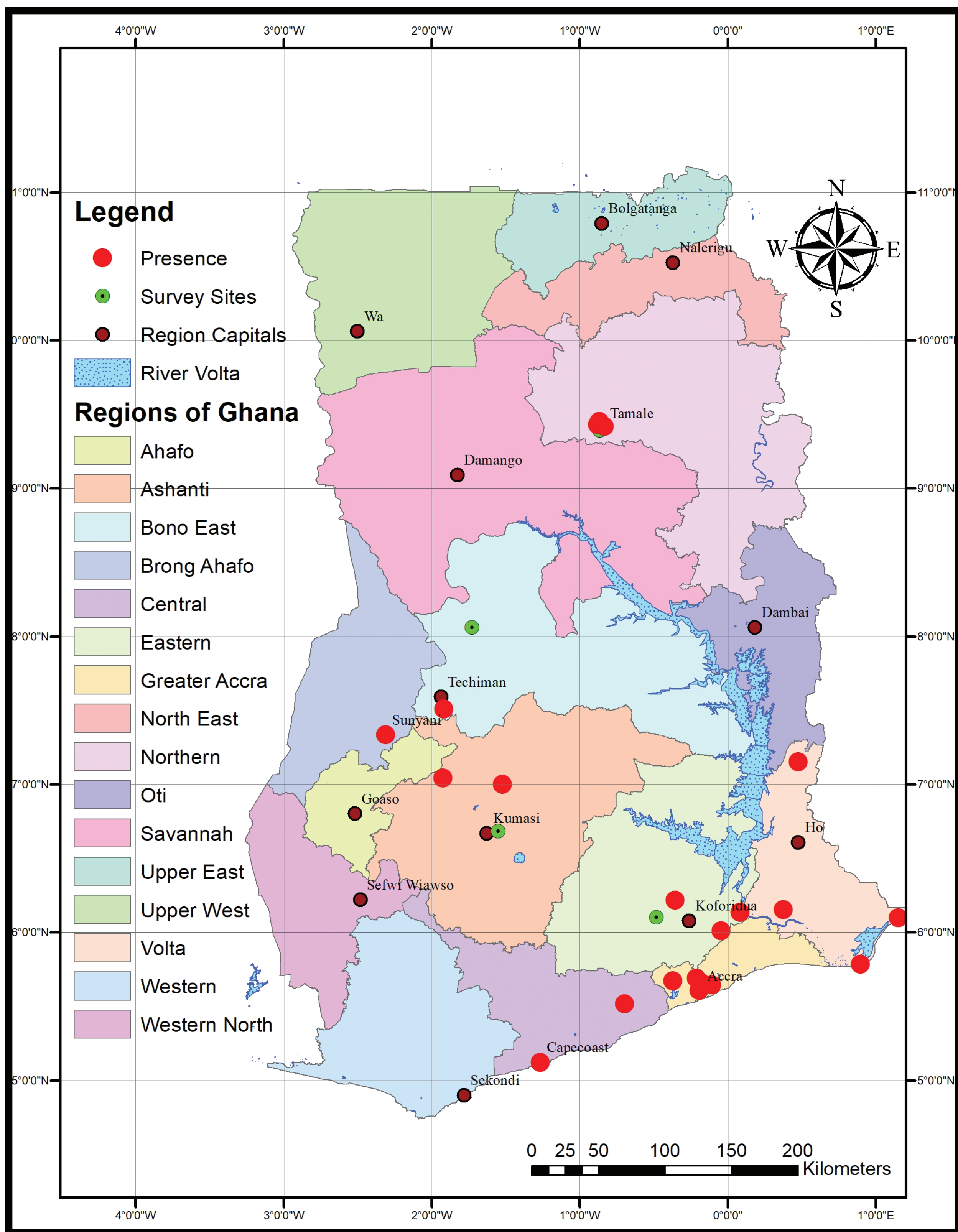

Fig. 5. Distribution map of *L. e. pseudobrassicae* in Ghana.

species identification. Keys by Blackman and Eastop (1984) (Table 2) were used to confirm aphids species identification. Data on the presence of aphids was collected and GPS points taken for all sampled regions. The mean number of aphids per plant (abundance) was utilized to compute the percentage regional abundance of *L. e. pseudobrassicae*.

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Table 3. Distribution and percentage regional abundance of *L.e. pseudobrassicae* and *M. persicae* in eight regions of Ghana

Region	Agro-ecological zone	Community	Presence	Mean num. of *L. e. pseudobrassicae* /plant	Mean num of *M. persicae* /plant	%RA of *L. e. p*
Greater Accra	Coastal Savanna	Dzorwulu	L	42	—	94.2
		Tema	LM	324	13	
		Agbogba	LM	212	35	
		Legon Univ. farm	L	206	—	
Eastern	Coastal Savanna and semi-deciduous forest	Kpong	LM	351	255	61.8
		Aseseeso	LM	292	192	
		Tafo	LM	63	14	
		Kyekyere	LM	176	87	
Northern	Guinea Savanna	Sangani	L	11	—	65.5
		Sawaba	M	—	24	
		Kpalesi	LM	14	10	
		Gurugu	LM	87	25	
Ashanti	Semi-deciduous forest	Akanfo	LM	173	48	78.6
		Opokukrom	LM	58	15	
Central	Coastal Savanna and semi-deciduous forest	Cape Coast	LM	60	28	69.2
		Agona Swedru	LM	30	12	
Volta	Coastal Savanna and semi-deciduous forest	Anloga	L	102	—	89.2
		Denu Lowcost	L	115	—	
		Hohoe	LM	71	32	
		Sogakope	LM	96	18	
Bono East	Semi-deciduous forest	Sunyani	LM	64	35	64.6
Brong-Ahafo	Transitional	Techiman	LM	275	110	71.4

L = presence of *L. e. pseudobrassicae*, M = presence of *M. persicae*, RA = regional abundance, – = aphids are absent.

Results and Discussion

Large colonies of dark green and pink aphids were observed on diseased cabbage plants examined in sites in and around Kpong and Kumasi, Ghana. DNA from sampled aphids was amplified by PCR using *CO I*-specific primers and PCR products sequenced. Of the 19 samples analyzed in this way, 15 were the generalist aphid, *Myzus persicae* and 4 were the brassica specialist *L. erysimi pseudobrassicae*. This marks the first detection of *L. e. pseudobrassicae* in Ghana. The pink colored aphid was identified as *Myzus persicae* (Sulzer) and the dark green was identified as *Lipaphis erysimi pseudobrassicae* (Davis) (Table 2, Figs. 2 and 3). *M. persicae* is already found on several crops, including cabbage, in Ghana (CIE 1979, Millar 1994.).

A BLAST search for homology using the generated sequences; Kpong 4 (GenBank MN267874), Kumasi 19 (GenBank MN267875), Aseseeso 51 (GenBank MN267876), and Kpong (GenBank MN267877) revealed 100% sequence similarity to *L. pseudobrassicae* isolated from cabbage plants in Iran (GenBank: MH183023.1) (Malvandi 2018). Pairwise sequence comparison of these sequences showed clear species demarcation between *L. e. pseudobrassicae* and *M. persicae* from individuals sampled in this study and reference sequences from GenBank (Fig. 4).

Examination of the morphology of the suspected *L. e. pseudobrassicae* aphids was carried out to confirm or refute the conclusion of the molecular analyses. The key morphological characters used for identification of mustard aphid (*L. e. pseudobrassicae*) are discussed as follows. Firstly aphids were found as large colonies on the lower leaf surface of infested cabbage plants and colonies possessed a barely visible thin layer of a white waxy secretion, although this may not be easily visible in the photograph shown (Fig. 2A). These colony properties are characteristic of this species (Kennedy et al. 1962, Adhab and Schoelz 2015). Observations of individual aphids showed that their cornicles (siphunculi) were not dark and are longer than the cauda and furthermore, the cauda were observed to be distinct and tongue-shaped, (Fig. 2A and B). Taken together, these are all distinguishing features of *L. erysimi* group (Kennedy et al. 1962). For *M. persicae*, the cornicles had the same color as the body; and were longer, >2 times the length of the cauda. Unlike *L. e. pseudobrassicae*, the cauda of *M. persicae* is cone-shaped (Fig. 3B).

Widespread infestation of cabbage is mostly attributed in the literature to *Lipaphis erysimi* (Kaltenbach) or to *L. pseudobrassicae* (Davis (Müller 1986). According to Müller (1986), the incidence of *Lipaphis* on cabbage has been reported from warm countries. Due to the absence of clear morphological distinguishing characters between *L. erysimi* and *L. pseudobrassicae*, biological or molecular tests may be appropriate for delimiting these two subspecies (Müller 1986, Hebert et al. 2003). Only one of the three subspecies (*L. erysimi*, *L. pseudobrassicae* and *L. alliariae* F. P. Müller) of the *Lipaphis* group, i.e., *pseudobrassicae*, can be considered a noxious insect, and its exact denomination must be used (Müller 1986). Although, in the Review of Applied Entomology the names *erysimi* as well as *pseudobrassicae* are used in connection with its occurrence on cabbage and mustard (Müller 1986). Paddock (1915) referred to this subspecies as *Aphis pseudobrassicae* (Davis), where he offered a detailed account of its biology in Texas (Eastop 1977, Blackman and Eastop 1984) and Essig (1948) and Eastop (1977) referred to *Lipaphis erysimi* as being synonymous to *Rhopalosiphum pseudobrassicae* (Davis) and it is known to be a vector of viruses on cabbage. It is alleged that the European form of *L. erysimi* is not normally a pest of cabbage (Cottie 1953, Blackman and Eastop 1984). Blackman and Eastop (1984) stated that *L. erysimi* (Kaltenbach) is synonymous to *R. pseudobrassicae*. This attests to the fact that the two subspecies are morphologically indistinguishable.

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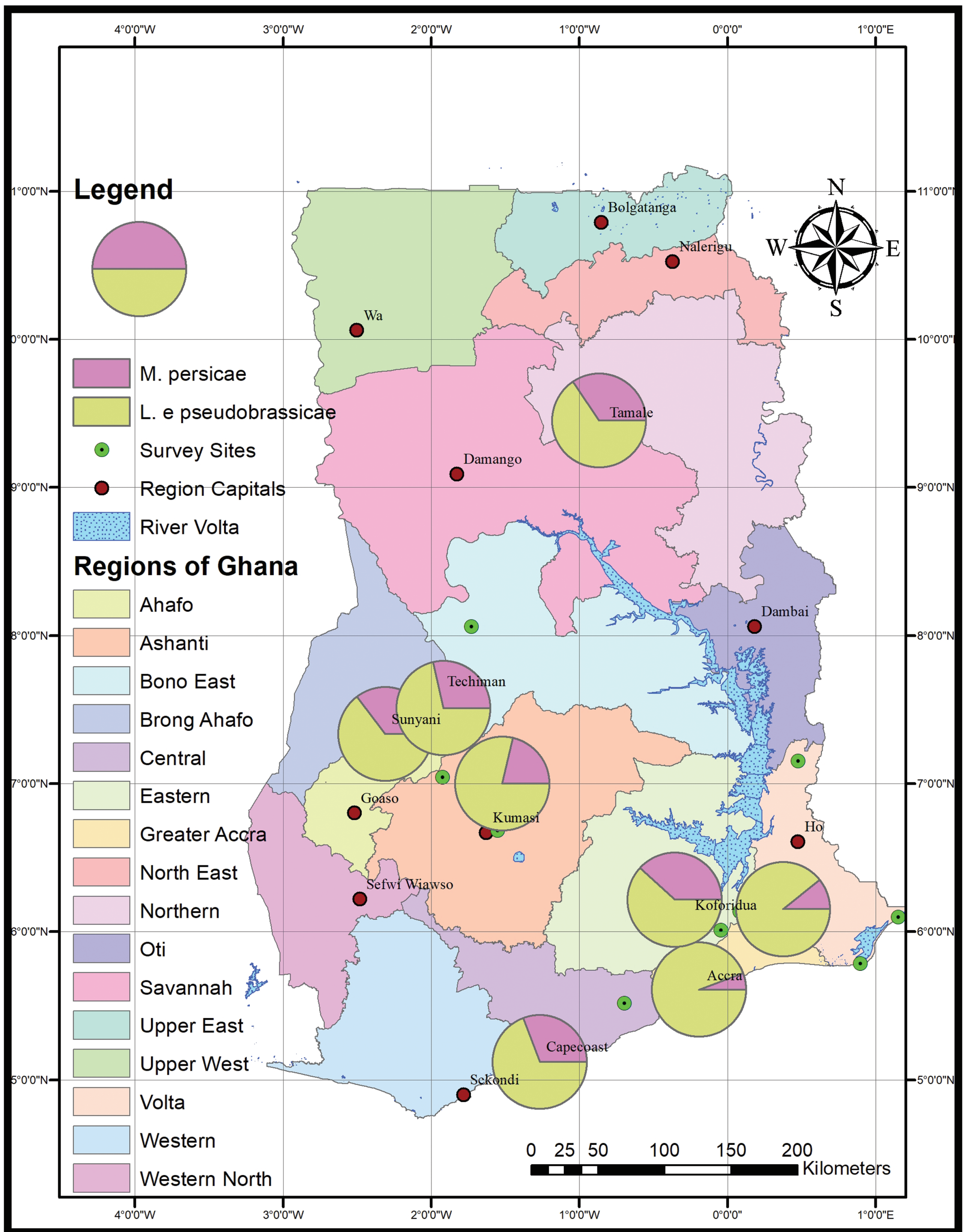

Fig. 6. Regional abundance of *L. e. pseudobrassicae* compared to *M. persicae* on cabbage in Ghana.

It is quite obvious that most of the reports of *L. erysimi*, are likely due to the presence of the subspecies *pseudobrassicae*, which is associated with serious damage on cabbage and mustard, and which is also a vector for the transmission of viral diseases, especially in Asia and Africa. Müller (1986) attests to this viewpoint, where he stated that he is aware of only a single report of *erysimi* on

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cabbage in Europe (Denmark) and that it is possible that this Danish report in reality concerns *pseudobrassicae* that immigrated from the Mediterranean area.

The *L. erysimi* group has been reported to be widely distributed in South Africa, Kenya, Benin, Mali, Sudan, Egypt, China, India, Vietnam, United States, Sweden, and many other countries, causing devastating losses in the production of cruciferous crops (Daiber 1971, Müller 1986, Verma and Singh 1987, Ronquist and Ahman 1990, Buntin and Raymer 1994, Begum 1995, Liu et al. 1997; James et al. 2010, Saethre et al. 2011, Jessie 2013, Adhab and Schoelz 2015, Tran et al. 2016). This is the first formal report of the mustard aphid, *L. e. pseudobrassicae* identified on cabbage in Ghana. The correct identification of *L. e. pseudobrassicae* and *M. persicae* is crucial for any planned implementation of control measures.

Farms sampled during the survey ranged from backyard gardens to large commercial farms. The majority of the farmers cultivated cabbage in urban and peri-urban areas to meet the demands of the readily available market in urban areas. Obuobie et al. (2006) reported similar findings; most exotic leafy vegetables are grown in and around urban areas to meet the ever-increasing urban demand.

In all the regions surveyed, *L. e pseudobrassicae* was present co-existing with *M. persicae* suggesting that all the regions have a suitable habitat for the establishment of the mustard aphid (Fig. 5). This further shows that this pest is highly adaptable to different types of environments as found in the different sampling areas. This finding is congruent with studies by Souza et al. (2017) who showed by laboratory studies that *L. pseudobrassicae* has better adaptation to changing temperatures compared to *M. persicae*. The percentage abundance of the mustard aphid *L.e. pseudobrassicae* was higher in all the surveyed regions on cabbage compared to *M. persicae* (Table 3; Fig. 6). Nevertheless, the abundance was highest in the southern part of the country and this is possibly due to the fact that, this area is a hotspot for growth of exotic leafy vegetables such as cabbage. The cabbage growing periods also varies significantly between the northern and southern sectors of the country. The southern sector is characterized by a bimodal rainfall regime while the northern sector experiences a unimodal rainfall regime (Agbessenou et al. 2016), which accounts for the year round growing of cabbage in the Southern sector. Additionally, most farmers in the North grow cabbage during the dry season as earlier reported by Timbilla and Nyarko (2004), which was outside the period of survey. Since aphid populations are dependent on environmental factors such as temperature, relative humidity, and rainfall, their absence in certain areas was attributed to this effect and also to the management regimes on farmer's fields. However, the survey results indicate that *L. e pseudobrassicae* is the most abundant and major aphid pest of cabbage in Ghana (Fig. 6).

L. e. pseudobrassicae and *M. persicae* both occur on cabbage plants in Ghana and seem to be associated with a disease that is devastating to the cabbage crop in Ghana. This raises the possibility that in addition to causing severe direct damage the aphids may be vectoring a necrotic disease of cabbage suspected to be of viral etiology (Fening et al. 2016).

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