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Host matrix has major impact on the morphology of *Pseudoperonospora cubensis*

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Abstract Oomycetes contain some of the economically most important pathogens of flowering plants. Most have a rather narrow host range, often being restricted to single host species. In downy mildews and other obligate biotrophic plant parasites, like powdery mildews and rusts, delimitating species on grounds of morphological characteristics is often hardly possible and thus often based on only subtle differences. This has led to the widespread application of a broad species concept for these organisms. Consequently, despite the fact that morphological differences were reported for *Pseudoperonospora cubensis* from different host species, the corresponding new pathogen species were not accepted as being independent, and the host range of *Pseudoperonospora cubensis* is reported to encompass more than 50 host species in the Cucurbitaceae in temperate to tropical climates. However, recent studies have reported narrow host ranges for other downy mildew genera and advocated a narrow species concept. Here, we report successful colonisation of five different tribes of the Cucurbitaceae by a strain of *Pseudoperonospora cubensis* and demonstrate that the host matrix has a major impact on the morphology of the pathogen. On the basis of five morphological criteria significant differences could be found for all hosts. These differences were more pronounced in phylogenetically unrelated than in related hosts. Our results provide evidence for a broad host range of *Pseudoperonospora cubensis* and demonstrate that species delimitation based on morphological characters is not feasible in *Pseudoperonospora* on Cucurbitaceae. Also in other biotrophic plant pathogens, the situation could be similar, thus necessitating thorough morphological, molecular phylogenetic and cross inoculation experiments for species recognition.

Keywords Cucurbit downy mildew · Obligate biotrophic plant pathogens · Host-pathogen interaction · Cucurbitaceae · Peronosporaceae · Species concepts

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Introduction

Oomycetes are fungal-like organisms that are unrelated to the Mycota, but belong to the kingdom Straminipila (Dick 2001), which also includes seaweeds and diatoms. The most species-rich order of the oomy-

cetes is the order Peronosporales, which contains mainly plant parasitic species, but also some fungus and animal pathogens. The largest family are the Peronosporaceae, which include the hemibiotrophic genus *Phytophthora* and the obligate biotrophic downy mildews. According to Voglmayr (2008) and Thines et al. (2009a) 18 genera are included in the Peronosporaceae with more than 800 described species. Among these, several cause economically important diseases of crops, including *Bremia lactucae* (lettuce downy mildew), *Plasmopara halstedii* (sunflower downy mildew), *Plasmopara viticola* (grape downy mildew), and *Pseudoperonospora cubensis* (cucurbit downy mildew). Earlier reports, stating that downy mildews have narrow host ranges, often limited to a single host species (Gäumann 1918, 1923; Crute 1981; Brandenburger 1985), have recently been confirmed by molecular phylogenetic studies (Cunnington 2006; Choi et al. 2007a, 2009a; García-Blázquez et al. 2008; Thines et al. 2009b). However, there is some evidence that a few downy mildew species are able to parasitise a variety of distantly related host species (Choi et al. 2005; Göker et al. 2009), similar to the situation described for the obligate biotrophic oomycete *Albugo candida* (Choi et al. 2007b, 2008, 2009b; Thines et al. 2009c), which causes white blister disease on a broad range of Brassicaceae, Capparaceae and Cleomaceae. As with rusts and in powdery mildews, in downy mildews it is often almost impossible to distinguish species on the basis of morphological characters. Therefore, before molecular phylogenetic investigations revealed the evolutionary diversity of these organisms, a broad species concept was usually applied, and some specialised forms were assumed to be present in some host families (Yerkes and Shaw 1959; Skidmore and Ingram 1985). One of these broad species is *Pseudoperonospora cubensis*, which is one of the most important and devastating diseases in cucurbitaceous crops. However, contrary to *Hyaloperonospora parasitica* (Göker et al. 2009) and *Peronospora farinosa* (Choi et al. 2007a), its integrity has not been questioned, but rather been confirmed by molecular phylogenetic studies (Choi et al. 2005). *Pseudoperonospora cubensis* has a worldwide distribution and is present in all cucurbit growing areas (Holmes et al. 2004). The host range of *P. cubensis* is reported to include over 50 cucurbits, among which are the following crop species—*Benincasa hispida, Citrullus lanatus*, *Cucumis sativus, Cucumis melo, Cucurbita pepo, Cucurbita maxima, Lagenaria siceraria*, and *Luffa cylindrica* (Lebeda and Widrlechner 2003; Thomas 1986). Although Sawada (1931) introduced some new segregate species of *Peronoplasmopara* (a synonym of *Pseudoperonospora*) parasitic to Cucurbitaceae on the basis of morphological differences, these were not considered different species by subsequent authors (Iwata 1942, 1953; Palti and Cohen 1980; Waterhouse and Brothers 1981), also because it was unclear if the variation observed might have been due to environmental factors. Dudka et al. (2007) examined the dependence of the sporangial dimensions of *Peronospora alta* on climatic conditions and assumed that humidity has a major impact on sporangial dimensions, and Iwata (1942) showed that the morphology of sporangiophores of *P. cubensis* varies with different temperatures. Waterhouse and Brothers (1981) focused their investigations on measurements of the sporangia of *P. cubensis* and found a significant influence of the host plant in this character. Delanoe (1972) reported that there are differences in morphology of sporangiophores and sporangia of *Plasmopara halstedii* isolated from different parts of the host plant. The investigations of Waterhouse and Brothers (1981) that demonstrated an impact of the host matrix on the morphology of sporangia were not followed up by investigations using a more comprehensive set of hosts and additional characters. Also, it is unclear, if climatic factors such as light, humidity and temperature were tightly controlled in their experiments.

The aim of this study was to investigate the potential dependence of the morphology of plant pathogens on the host matrix, using the example of *P. cubensis*. These investigations might shed light on the question of whether it is justified to erect new species or confirm previously described species solely on the basis of morphological characters and host matrix, which has been common practice for many plant pathogens. *Pseudoperonospora cubensis* was chosen for these investigations, because of its reported broad host range. For evaluating these reports, and for investigating the impact of the host matrix on the morphology of the pathogen, six species belonging to five different tribes of the Cucurbitaceae (Kocyan et al. 2007), were used in this study—*Cucurbita maxima*, *Citrullus lanatus* and *Cucumis sativus*, *Sicyos angulatus*, *Luffa cylindrica*, and *Bryonia dioica* belonging to the tribes Cucurbiteae, Benincaseae, Sicyeae, Luffeae, and Bryonieae respectively.

Material and methods

Inoculation experiments were carried out using the *P. cubensis* strain P.C. 26/01 that originated from the Olomouc Region of the Czech Republic. The strain was originally isolated from *Cucumis sativus* and is maintained on *C. sativus* at the Institute of Botany of the University of Hohenheim in climate chambers (16°C, 14 h light, 10 h darkness). Inoculations for continuous cultivation of P.C. 26/01 and cross-inoculations were done using a dab-off technique as described previously (Runge and Thines 2009). For inoculations, the lower surface of uninfected leaves was moistened with deionised water. Subsequently, sporulating leaf-parts were gently dabbed onto the moist leaf surface of the uninfected leaf several times, touching previously unaffected areas of the leaf for inoculation. The dab-off technique leads to different spore concentrations over the leaf surface. Thus, the spore concentration ranges from areas without sporangia to areas with very high amounts of inoculum. This technique has the advantage that optimal inoculum concentrations, which will differ from species to species and even from cultivar to cultivar, need not be determined, because these will be met through the differences in inoculum load throughout the leaves. Fully mature leaves of *Cucurbita maxima*, cultivar "Kaempe Melon", *Citrullus lanatus*, cultivar "Sugar Baby", *Cucumis sativus*, cultivar "Chinese Slangen", *Sicyos angulatus*, *Luffa cylindrica*, and *Bryonia dioica* were taken from plants in 5 to 10-leaf stage grown in greenhouses at the University of Hohenheim, except for the leaves of *S. angulatus*, which were cut from outdoor plants (Botanical Garden of the University of Hohenheim), while care was taken to select leaves of comparable age. After inoculation the leaves were transferred to transparent boxes (approximately 30 cm long, 20 cm wide, 5 cm high) on water-soaked paper towels to ensure that 100% relative humidity (RH) was maintained within the boxes. Cross-inoculations were done in three technical replicates. Cross inoculations using *Bryonia dioica* and *Sicyos angulatus* were repeated for testing reproducibility over time. As these tests were successful, infection trials for the other cucurbitaceous hosts were done only once, in three replicates carried out at the same time. Two days after first sporulation was observed sporangiophores were picked from the leaf surface with precision tweezers, transferred to a drop of water on a microscopic slide and covered with a coverslip. The morphology of sporangia and sporangiophores were investigated using a Biomed (Leitz, Wetzlar, Germany) light microscope. Pictures of sporangia and sporangiophores were taken using a Canon PowerShot A640 photocamera (Canon, Tokyo, Japan). Before each picture series a picture of a stage micrometer was taken to calibrate the measurements, which were conducted using the AxioVision LE software (Carl Zeiss Imaging Solutions, München, Germany). The characters examined were the length of the sporangiophores (n=25), the length of the ultimate branchlets (n=100), and the length and the width of the sporangia (n=100). The fifth character, the ratio of length to width of the sporangia was calculated for each individual sporangium. Subsequently, the data were statistically analysed using STATISTICA '99 software (StatSoft, Tulsa, OK, USA). The Mann-Whitney-*U*-Test (Mann and Whitney 1947) was applied for investigating the significance of the morphological differences of the pathogen on the host species investigated.

Results

Four to eight days post inoculation, first sporulation could be observed (*Sicyos angulatus*: 4 d; *Cucurbita maxima*, *Cucumis sativus*: 5 d; *Luffa cylindrica*: 6 d; *Bryonia dioica*: 7 d; *Citrullus lanatus*: 8 d). The parasitised leaves with the respective symptoms are shown in Fig. 1. In the case of *Cucurbita maxima* (Fig. 1a) some areas became necrotic. In these necrotic areas, sporangiophores occurred in sparse groups. Also on the leaves of *Citrullus lanatus* (Fig. 1b) and *Luffa cylindrica* (Fig. 1e) there was only sparse sporulation, with sporangiophores dispersed throughout almost round, chlorotic to necrotic spots. In case of *B. dioica* (Fig. 1f) round chlorotic to necrotic spots could also be observed. In contrast to the former two hosts the sporulation was dense on leaves of *Bryonia dioica*, with the result that the infection spots were exhibiting a blackish colour. In case of *Sicyos angulatus* (Fig. 1d) the sporulation was restricted to few areas; however, in these areas the sporulation was very profuse and therefore appeared floccose and dark grey in colour. On *Cucumis sativus* (Fig. 1c), the native host of the isolate, sporulation occurred on almost the whole lower leaf surface and

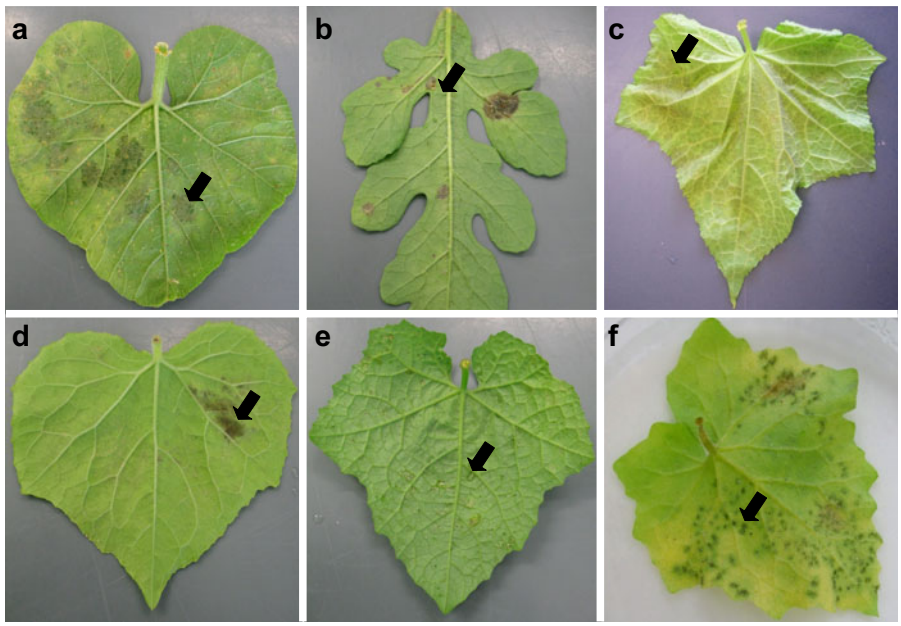

Fig. 1 Symptoms of *P. cubensis* (black arrows) on the parasitised leaves of the host plants *Cucurbita maxima* (**a**), *Citrullus lanatus* (**b**), *Cucumis sativus* (**c**), *Sicyos angulatus* (**d**), *Luffa cylindrica* (**e**) and *Bryonia dioica* (**f**)

some parts became densely covered with sporangiophores, resulting in a downy and greyish appearance. In all replicates the symptoms on the respective hosts were similar. No differences in morphology and shape of the fully mature sporangiophores and sporangia from the different replicates for a specific host could be observed.

The morphological characterisation of *P. cubensis* from the different hosts is given in Table 1, and the results of the statistical analyses of the morphology on different hosts are summarised in Fig. 2. *Pseudoperonospora cubensis* differed in almost all characters in at least one of the alternative hosts markedly in morphology compared to its original host, as well as in cross comparisons. In the analysis of the length of the sporangiophores two groups were apparent. One included *Cucurbita maxima*, *Citrullus lanatus*, and *Bryonia dioica* with mean sporangiophore lengths of 289 μm, 301 μm, and 321 μm, respectively, and no significant differences compared to each other. The second group included *Cucumis sativus*, *Sicyos angulatus*, and *Luffa cylindrica* with mean lengths of 387 μm, 377 μm, and 393 μm, respectively. When comparing members of the first group with the ones of the second group, significant differences were observed in each possible combination. In the length of the ultimate branchlets, only *Bryonia dioica* with a mean of 9.45 μm showed significant differences to all other hosts (*Cucurbita maxima*: 8.33 μm, *Cucumis sativus*: 8.35 μm, *Sicyos angulatus*: 8.74 μm, *Luffa cylindrica*: 8.18 μm), except for *Citrullus lanatus*, which was exhibiting an intermediate mean length of 9.19 μm and therefore showed significant differences to none of the others. On the basis of the length of the sporangia *Citrullus lanatus*, *Cucumis sativus*, and *Luffa cylindrica* formed a homogeneous group (mean lengths of 21.6 μm, 21.1 μm, and 21.7 μm, respectively). Sporangia taken from *Cucurbita maxima* and *Bryonia dioica* were longer (23.4 μm and 24.1 μm, respectively) and showed no significant differences to each other. Shortest sporangia were found in *Sicyos angulatus* with a mean of 19.5 μm. Differences between the three groups were highly significant ($p<0.001$). In the width of sporangia *Cucurbita maxima*, *Citrullus lanatus*, and *Bryonia dioica* (means of 16.0 μm, 15.5 μm, and 15.7 μm, respectively) formed a group with no internal significant differences. Also *Cucumis sativus* (mean 15.4 μm) was not significantly different from *Citrullus lanatus*. But all other combinations (*Sicyos angulatus*: mean of 13.1 μm, *Luffa cylindrica*: mean of 16.5 μm) showed significant deviation. The shape of the sporangia, expressed by the ratio of length to width, revealed that *Sicyos angulatus* did not differ significantly in shape from *Cucurbita maxima* and *Bryonia dioica* (means of 1.49, 1.47, and 1.54, respectively), but taking into account their length and width it is apparent that sporangia on *Sicyos angulatus* were significantly

Table 1 Morphological characteristics of *P. cubensis* on different Cucurbitaceae

Host species			
	Cucurbita maxima	*Citrullus lanatus*	*Cucumis sativus*
Sporangiophore length	(205-)244–289–345(-425)	(153-)226–301–369(-397)	(186-)280–387–494(-606)
Length of ultimate branchlets	(3.08-)6.26–8.33–10.8(-16.2)	(3.63-)6.11–9.19–13.8(-18.4)	(2.93-)6.03–8.35–11.3(-15.2)
Sporangial length	(13.6-)21.2–23.4–25.9(-32.9)	(14.3-)19.6–21.6–23.4(-27.1)	(14.2-)19.0–21.1–23.6(-30.1)
Sporangial width	(11.7-)14.5–16.0–17.4(-23.4)	(10.0-)13.9–15.5–17.1(-19.7)	(10.3-)14.3–15.4–18.0(-21.5)
Ratio length to width of sporangia	(1.01-)1.30–1.47–1.62(-1.84)	(1.01-)1.31–1.41–1.52(-1.88)	(1.00-)1.22–1.38–1.47(-1.77)
	Sicyos angulatus	*Luffa cylindrica*	*Bryonia dioica*
Sporangiophore length	(214-)296–377–483(-570)	(253-)314–393–439(-500)	(198-)258–321–429(-465)
Length of ultimate branchlets	(2.45-)6.29–8.74–12.9(-18.7)	(2.11-)5.04–8.18–9.74(-14.7)	(4.91-)7.42–9.45–12.9(-18.0)
Sporangial length	(12.0-)16.3–19.5–21.2(-27.1)	(16.3-)19.4–21.7–23.5(-30.1)	(18.8-)21.8–24.1–27.1(-32.4)
Sporangial width	(9.78-)12.1–13.1–14.2(-17.5)	(12.2-)14.8–16.5–18.1(-21.4)	(11.6-)14.8–15.7–16.9(-20.1)
Ratio length to width of sporangia	(1.01-)1.18–1.49–1.59(-1.80)	(1.03-)1.23–1.32–1.42(-1.68)	(1.25-)1.44–1.54–1.64(-1.95)

All measurements given in the form (minimum-) border of 30%—*mean*—border of 30% (-maximum). All values rounded to three counting digits

smaller. *Cucurbita maxima* and *Bryonia dioica* showed weakly significant ($p<0.05$) differences in the shape of sporangia. The shape of the sporangia of *Cucumis sativus* and *Citrullus lanatus* (1.38 and 1.41, respectively) was not significantly different rendering it impossible to distinguish sporangia from these hosts. All of the other combinations showed significant differences. Sporangia from *Luffa cylindrica* were, with a ratio of 1.32, more globose than sporangia from all other hosts.

A graphical display of all significant differences of the respective host-combinations at different *p*-levels (Fig. 3) illustrates that only two combinations, *Cucurbita maxima-Bryonia dioica* and *Citrullus lanatus-Cucumis sativus*, had no highly significant differences. The combination *Cucurbita maxima-Citrullus lanatus* showed only one highly significant difference. On the other hand the *Luffa cylindrica-Bryonia dioica*-combination showed highly significant differences in four of the five characters investigated. The *Citrullus lanatus–Cucumis sativus*-combination was the sole one with a single significant difference only in all characters investigated. Except for this combination, all other combinations had at least two significant differences at a *p*-value level below 0.05. *Pseudoperonospora cubensis* from *Bryonia dioica* had overall the most statistically supported differences (18 of 25 possible), especially due to the longest ultimate branchlets and the longest and most elongated sporangia on this host. Also *P. cubensis* from *Luffa cylindrica* revealed many supported differences to other hosts in several characteristics (17), in particular due to the shortest ultimate branchlets, the broadest and roundest sporangia, and the longest sporangiophores on this host compared to the other hosts. Also *P. cubensis* from *Sicyos angulatus* proved to be equally divergent in morphology. *Pseudoperonospora cubensis* from *Citrullus lanatus* was the host combination with the fewest differences to others, because most characters investigated were close to the mean of the other isolates.

Discussion

First sporulation was observed after 4–8 days post inoculation. It is supposed that the first sporulation reflects the sporulation time under optimal inoculum load, as inoculations were done using the dab-off technique, which ensures a heterogeneous inoculum

a - Length of sporangiophores	*B. dioica*	*L. cylindrica*	*S. angulatus*	*C. sativus*	*C. lanatus*	*C. maxima*
Cucurbita maxima						X
Citrullus lanatus					X	0,25826
Cucumis sativus				X	0,00394	0,00076
Sicyos angulatus			X	0,63760	0,00714	0,00070
Luffa cylindrica		X	0,47539	0,79194	0,00006	0,00000
Bryonia dioica	X	0,00099	0,01698	0,01386	0,40223	0,11612

b - Length of ultimate branchlets	*B. dioica*	*L. cylindrica*	*S. angulatus*	*C. sativus*	*C. lanatus*	*C. maxima*
Cucurbita maxima						X
Citrullus lanatus					X	0,22008
Cucumis sativus				X	0,18718	0,99480
Sicyos angulatus			X	0,68205	0,41974	0,77943
Luffa cylindrica		X	0,57014	0,90112	0,13090	0,97497
Bryonia dioica	X	0,00826	0,04512	0,00996	0,29016	0,00714

c - Length of conidia	*B. dioica*	*L. cylindrica*	*S. angulatus*	*C. sativus*	*C. lanatus*	*C. maxima*
Cucurbita maxima						X
Citrullus lanatus					X	0,00004
Cucumis sativus				X	0,05220	0,00000
Sicyos angulatus			X	0,00012	0,00000	0,00000
Luffa cylindrica		X	0,00000	0,05572	0,97667	0,00003
Bryonia dioica	X	0,00000	0,00000	0,00000	0,00000	0,26125

d - Width of conidia	*B. dioica*	*L. cylindrica*	*S. angulatus*	*C. sativus*	*C. lanatus*	*C. maxima*
Cucurbita maxima						X
Citrullus lanatus					X	0,13863
Cucumis sativus				X	0,30914	0,00490
Sicyos angulatus			X	0,00000	0,00000	0,00000
Luffa cylindrica		X	0,00000	0,00000	0,00039	0,01433
Bryonia dioica	X	0,00027	0,00000	0,02697	0,60638	0,25377

e - Ratio length to width of conidia	*B. dioica*	*L. cylindrica*	*S. angulatus*	*C. sativus*	*C. lanatus*	*C. maxima*
Cucurbita maxima						X
Citrullus lanatus					X	0,01122
Cucumis sativus				X	0,15830	0,00010
Sicyos angulatus			X	0,00000	0,00001	0,20117
Luffa cylindrica		X	0,00000	0,00066	0,00001	0,00000
Bryonia dioica	X	0,00000	0,59028	0,00000	0,00000	0,01440

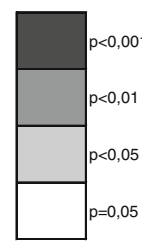

Fig. 2 Statistical analysis of the measurements of morphological characters of *P. cubensis* isolated from different hosts. The numbers in the lower right matrices are p-values for the significance of morphological differences of *P. cubensis* on the respective hosts. The p-levels are visualised by different shadings as given in the legend in the lower right corner

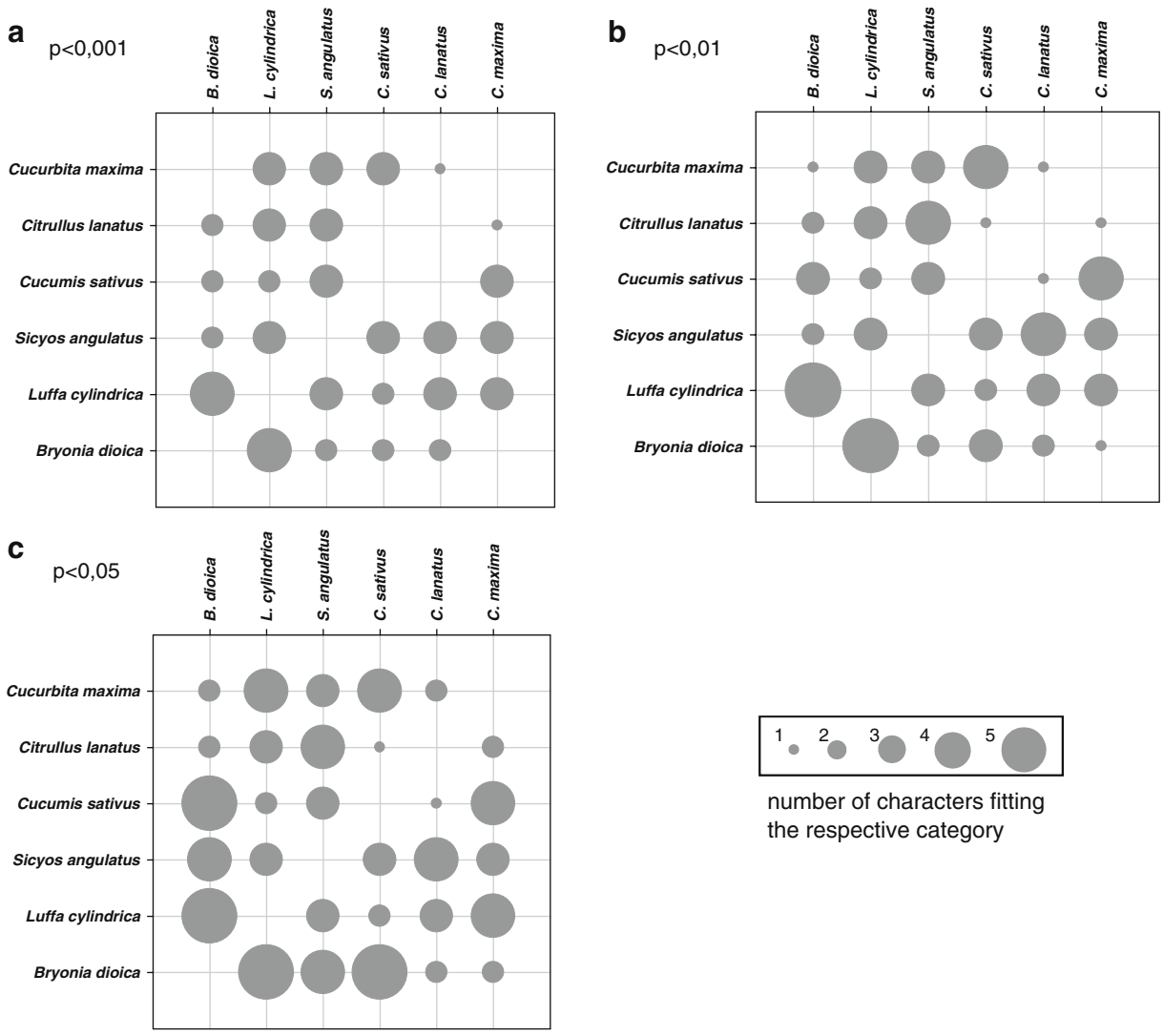

Fig. 3 Graphic display of the distinctiveness of the morphology of *P. cubensis* on various hosts. Number of differences in all characters investigated for the respective host-pathogen combinations with the respective p-levels are visualised by the size of the grey circles

load over different areas of the leaves. Usually *P. cubensis* produces a new generation of sporangiophores and spores 7–8 days past inoculation (Urban and Lebeda 2007). The fact that in our investigations sporulation occurred already after 5 days on *C. sativus* supports previous results of the presence of distinct physiological forms of *P. cubensis* (Shetty et al. 2002; Lebeda and Widrlechner 2003). Our finding that symptoms and onset of the disease differed widely on the different hosts investigated provide further evidence for the existence of a broad genetic variation in resistance to *P. cubensis* among cucurbits as discussed by Lebeda (1991) and Lebeda and Widrlechner (2004).

Recent molecular phylogenetic studies have shown that the broad species concept advocated by Yerkes and Shaw (1959) is not tenable for most downy mildews (Riethmüller et al. 2002; Voglmayr 2003; Voglmayr et al. 2004; Cunnington 2006; Choi et al. 2007b, 2009b; Göker et al. 2009; Thines et al. 2009b). For most of the species of the downy mildews, it is hardly possible to distinguish between phylogenetically related species based on morphological characters, but thorough morphological investigations in *Peronospora* species from Lamiaceae have revealed differences that enable species delimitation in the *Peronospora lamii* species complex (Choi et al. 2009b; Thines et al. 2009b).

However, contrary to the situation in other downy mildew genera affecting multiple species within a specific host family, genetic distances within *P. cubensis* are very short (Choi et al. 2005), which hinders easy molecular phylogenetic delimitation of species that might be present in *Pseudoperonospora* parasitic to Cucurbitaceae. But as short genetic distances do not necessarily rule out the presence of independent species, multigene analyses are often necessary for distinguishing closely related species (Blair et al. 2008). In addition, morphological investigations and cross-inoculations are necessary for clarification. However, the broad host spectrum of the *P. cubensis* strains previously characterised (Lebeda 1991; Shetty et al. 2002; Lebeda and Widrlechner 2003, 2004) and confirmed in this study using the strain P.C. 26/01, casts doubts on the credibility of the species segregated from *P. cubensis* on the basis of host matrix and morphological differences by Sawada (1931). However, in previous studies characterising the infectivity of *P. cubensis* strains on various hosts, potential differences in the morphology of the strains used were not investigated. Therefore, the morphological species introduced by Sawada (1931), which were not considered independent species in comprehensive treatises (Iwata 1942, 1953; Palti and Cohen 1980; Waterhouse and Brothers 1981), could still be credible, even when considering possible variations caused by differing temperatures or humidity (Iwata 1942; Dudka et al. 2007). However, this study demonstrates that the morphology of the pathogen is largely dependent on the host matrix. This is in line with the results of Waterhouse and Brothers (1981), who focused on sporangial dimensions on selected hosts and found significant differences between these hosts. To exclude both temperature and humidity effects, we used controlled conditions for both parameters and also applied identical light conditions after inoculation. Cohen (1981) suggested that the sporangial yield per unit area of affected leaf tissue in a moist chamber depends upon many factors, the most important of which were plant species and variety, age and size of lesions, nutritional status of the host, temperature, and illumination. However, investigations using *Cucmis melo* as a host showed that the variation in nutrient solutions did not affect the size of sporangia (Bains and Jhooty 1978). An influence of the organ type infected is likely; as Delanoe (1972) pointed out that there are significant differences in morphology of sporangiophores and sporangia of *Plasmopara halstedii* isolated from different parts of sunflowers. Therefore, only fully mature leaves in comparable stages of development were used in this study. It is most likely due to highly homogenous sporulation conditions applied in this study that the influence of the host matrix on the morphology of the pathogen could be demonstrated clearly, and it should be taken into consideration that for field material the variation in the morphological characters is expected to be much higher. The finding of Choi et al. (2005) that the morphology of sporangia and sporangiophores of *P. cubensis* were indistinguishable for all specimens from *Cucumis*, *Cucurbita*, and *Citrullus* investigated, could well be the result of a high variation caused by different sporulation conditions (like temperature and humidity), leaf ages or organ affected. Unfortunately, no statistical analyses were presented to underscore their results.

When comparing the morphological results of this study in the light of the molecular phylogeny of the Cucurbitaceae presented by Kocyan et al. (2007), some patterns were becoming apparent. *Bryonia dioica* belonging to the Bryonieae is the most basal member of the Cucurbitaceae investigated here, and its phylogenetic distance to the other hosts used in this study is large. Interestingly, this is also the host on which *P. cubensis* shows the most divergent morphology when compared to the other hosts. *Luffa cylindrica* and *Sicyos angulatus*, belonging to the Luffeae and Sicyeae respectively, the hosts with the second most significant differences, also occupy rather basal phylogenetic positions. *Citrullus lanatus* and *Cucumis sativus*, the hosts on which the morphology of the pathogen was found to be almost identical, belong to the same tribe, the Benincaseae. *Cucurbita maxima* of the sister tribe Cucurbiteae occupied an intermediate position, both in the phylogenetic distance from the native host (*Cucumis sativus*) of the isolate used in this study and in morphological differences induced in the pathogen compared to other hosts. Thus, it could be suggested that the phylogenetic distance of the hosts correlates with the morphological differences induced in a pathogen, with greater distances between hosts being more likely to induce significant differences in pathogen morphology. However, at present this can only be regarded as a tendency and further investigations with a broader set of Cucurbitaceae will be necessary to clarify if the observed pattern follows a general rule. Considering the high variation of the sporangial

dimensions depending on the host matrix it seems highly unlikely that the species segregated from *P. cubensis* by Sawada (1931) are tenable. In addition to the sporangial dimensions, which were found to be influenced the most by the host matrix in this study, in line with results of Waterhouse and Brothers (1981), our results provide evidence that the morphology of sporangiophores can also be influenced by the host matrix. Further investigations are necessary to clarify which morphological characters are significantly influenced by the host matrix, and which are not. This will help clarifying, if variation observed in newly reported hosts could rather be the result of morphological plasticity of the corresponding character or due to the observation of an unknown species with deviating morphology.

Judging from our results, species of downy mildews described on the basis of morphological differences with no phylogenetic support, as with some *Plasmopara* species occurring on Apiaceae (Voglmayr et al. 2004), should be treated with caution and be verified by multigene analyses and cross-infection experiments in order to rule out a possible influence of the host matrix on the morphological differences observed. However, the possibility that two potential pathogen species may infect the same host species cannot be the sole criterion for species rejection, as for both *Hyaloperonospora* (Göker et al. 2009) and *Plasmopara* (Komjáti et al. 2007) it is known that phylogenetically clearly distinct species are able to infect the same host species.

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