Do fungal spore morphological traits correlate with allergenicity?

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Abstract
Fungal spores, like pollen, are identified as sources of allergens, yet fungal spore morphology, unlike pollen morphology, has not been correlated with allergenicity. In this study, we listed allergenic fungi reported from published literature and gathered information about their spore morphologies including the species' lifestyle, the ability to produce mycotoxins, and the types of hypersensitivity reactions they induced. We tested the association of these spore traits with the hypersensitivity reaction through correspondence analysis with Chi-square as the measure of distance. Our research listed a total of 158 species of allergenic fungi belonging to 82 genera and 30 taxonomic orders. Most of the species (n = 122) elicited a Type I hypersensitivity reaction while 33 species had more than one hypersensitivity type (Types I-III-IV). The most common allergenic fungi belong to the genus *Alternaria* (41 species). Two fungal taxa commonly found in spoiled food, *Penicillium* (9 species) and *Aspergillus* (8 species), were also listed as allergenic. We did not find any strong correlation between allergenic reaction with the following spore traits: shape, texture, color, size, appendages, and with the type of spores, presence of mycotoxins, and the species lifestyle. However, spore length and width were positively associated with hypersensitivity reaction. Allergenic fungi with short and/or narrow spores can likely cause multiple types of hypersensitivity reactions while fungi with large and/or wide spores can induce either Type I or Type III hypersensitivity reaction. Our research study provides interesting insights into the role of fungal spore morphologies in human allergenicity.

Keywords: allergens, allergenic fungi, hypersensitivity reaction, spore morphology, systematic review

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Introduction

The prevalence of allergies is increasing globally, thereby affecting millions of individuals and approximately 80 % of families (Sánchez-Borges et al. 2018). Its cause ranges from dust, pollens to fungal spores, exposure to chemicals from plants and secretions from animals and insects to drugs, and ingestion of food allergens such as nuts and grains (Schnyder and Pichler 2009, Woodfolk et al. 2015, Goodman et al. 2020). From these allergens, 40 % of the world population is sensitized to pollen, leading to the common allergic rhinitis (Depciuch et al. 2016). Pollen morphology has been correlated with allergenicity, with the smaller pollen size being significant in causing the allergenic reaction. Their small size provides a greater advantage for wind dispersal, thereby increasing the pollen concentration in the air and the possibility of entering the human airways (Diethart et al. 2007, Pablos et al. 2016). In the Philippines, Sabit et al. (2020) stated that pollen allergens affect about 20 % of the Philippine population.

Another common allergen is the fungal spores. Fungi are the most abundant microorganisms in the atmosphere as their spores are easily dispersed and could arise from events and environments associated with human activities (Ortega-Rosas et al. 2019). Interestingly, the quantity of fungal spores in the atmosphere exceeds that of pollen by a thousand-fold and it can remain prevalent in the air throughout the year with seasonal peaks (Burge 2002, Boddy 2016). Fungal spores are generally smaller than pollen, making it more possible to reach the host's alveoli, and subsequently cause severe allergic reactions (Żukiewicz-Sobczak 2013). Other hypersensitivity reactions can also be triggered through ingestion or contact with these fungal spores (Simon-Nobbe et al. 2007). Since both indoor and outdoor fungi are involved in fungi-induced allergies, more than 80 fungal genera have been so far identified as allergenic which mainly induce Type I hypersensitivity (Kurup et al. 2000), albeit others can cause clinical manifestations based on Type III or Type IV hypersensitivity (Simon-Nobbe et al. 2007). However, unlike pollen, investigations on the potential contribution of fungal spore morphology to allergenicity remain scarce. This study, therefore, aims to correlate fungal spore morphology with allergenicity. We seek to delineate common fungal spore traits that may contribute to allergic reactions and serve as indicators of hypersensitivity-related risk assessment.

Materials and methods

Listing of allergenic fungi

We conducted a literature survey and systematic analysis in this study. We initially utilized the paper of Simon-Nobbe et al. (2007) as the primary reference to list the species of allergenic fungi (n = 143). We expanded the list by conducting a literature search with the keywords "allergenic fungi" and "allergenic fungal species listing" on online journal databases, e.g., JSTOR (jstor.org), the National Center for Biotechnology Information (ncbi.nlm.nih.gov), Science Direct (sciencedirect.com). From our search hits, non-related studies were excluded based on information on their titles and abstracts, and on the accessibility of the paper. We found twelve studies in addition to the primary reference of Simon-Nobbe et al. (2007) to list down the allergenic fungal species, i.e., Horner et al. (1995), Green et al. (2003), Kurup (2003), Knutsen et al. (2012), Crameri et al. (2013), Kochar et al. (2014), Oh et al. (2014), Fukutomi and Taniguchi (2015), Vijayakumar et al. (2017), Bhattacharya et al. (2018), Dey et al. (2018), and Songnuan et al. (2018). In case only the fungal genus is identified in the published study, an additional literature search on the same online databases was conducted with the genus name coupled with the keywords "allergenic species" or "allergy". Species names of the listed allergenic fungi were validated with Index Fungorum (2022).

Listing of fungal spore traits

We initially listed the set of spore morphologies and other characters for this study. These included (1) species lifestyle, (2) spore shape, (3) spore texture, (4) spore color, (5) spore size, (6) spore length, (7) spore width, (8) the type of spores, (9) the presence of spore appendages, and (10) the ability to produce mycotoxins. To determine the morphological characters for each species, we initially searched the traits from reference taxonomic monographs, i.e., the Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species (Watanabe 2010) and Larone's Medically Important Fungi: A Guide to Identification (Walsh et al. 2018). Additionally, we also searched online taxonomic databases, e.g., MycoBank (mycobank.org), Mycology Online at the University of Adelaide (adelaide.edu.au/mycology), and Doctor Fungus of Mycoses Study Group Education & Research Consortium (MSG ERC) (drfungus.org). In these databases, characters were searched by typing the species name. For other missing characters, we also conducted an extensive literature search by typing the species name coupled with the following keywords: 'spore morphology', 'species description', 'mycotoxins', 'lifestyle', and 'nutrition mode'.

Following listing of spore morphologies and other traits, these were categorized as follows: (**1**) *lifestyle*: biotrophic, endophytic, hemibiotrophic, necrotrophic, saprotrophic, and varying lifestyle for species that exhibits two or more lifestyles, following the definition of De Silva et al. (2016); (**2**) *spore shape*: elliptical [ellipsoidal or cylindrical appearance], fusiform [spindle-like shape], globose [spherical], ovate [oval outline], quadrilateral [four-sided figure], and variable [multiple shapes]; **(3)** *spore texture*: smooth [partially or completely smooth], rough [has a specific ornamentation], and smooth to rough; **(4)** *spore color*: light, dark, and light to dark; **(5)** *spore size*: I [long and wide], II [long and narrow], III [short and wide], and IV [short and narrow]; **(6)** *spore length*: long [>20 µm] and short [≤20 µm]; **(7)** *spore width*: wide [>10 µm] and narrow [≤10 µm]; **(8)** *type of spore*: asexual spore and sexual spore; **(9)** *presence of spore appendages*: absent and present; and **(10)** *ability to produce mycotoxins*: absent and present.

Identifying the types of hypersensitivity reaction (HR)

We also listed the type/s of hypersensitivity reactions for each of the listed allergenic fungi. The types follow Gell and Coombs's classification of hypersensitivity reaction for fungal allergies as described in the papers of Simon-Nobbe et al. (2007) and Sheldon et al. (2014). This is also supplemented by an extensive literature search to identify the type of hypersensitivity reaction elicited by each listed species. Species without any reported type of hypersensitivity reaction were excluded from the analysis and designated as potentially allergenic fungi.

Data analysis

From our listing of species, spore traits, and hypersensitivity reactions, we initially generated a table with the frequencies per category using the PivotTable tool of Microsoft Excel Version 2202. We did correspondence analysis (CA) in row principal normalization with Chi-square (χ^2) as the distance measure with IBM SPSS Statistics version 20. The spore traits were designated as the row variables while the types of hypersensitivity reaction were assigned as the column variables. For each character, statistical values from the χ^2 test of independence at a 5 % significance level, the Cramér's *V* coefficient, and a biplot were generated. To interpret the association patterns, spore traits within the same quadrant of a particular hypersensitivity reaction are deemed strongly associated. For spore traits with only two categories, a multiple correspondence analysis (MCA) with symmetrical normalization with χ^2 as the distance measure was conducted.

Results

Allergenic and potentially allergenic fungi. A total of 33 taxonomic orders, 101 genera, and 192 species of allergy-associated fungi were listed based on our literature analysis. Of these, 30 taxonomic orders (excluding Dacrymycetales, Geastrales, and Peronosporales) and 82 genera were confirmed as allergenic as indicated by their reported hypersensitivity reactions (Table 1). The excluded taxonomic orders had these three species, *Dacrymyces deliqeuscens* (Dacrymycetales), *Geastrum saccatum* (Geastrales), and *Plasmopara viticola* (Peronosporales), identified as potentially allergenic species but without any reported hypersensitivity reactions. We also identified 24 species as potentially allergenic as these were listed as allergenic fungi in published literature but with no reported hypersensitivity reactions (Table 3). Of these 24 taxa, 15 were identified only at the genus level, and hence, their species identity could not be ascertained. In addition, 10 species were reported as allergenic with Type I hypersensitivity reaction, but identification was limited only to the genus level (Table 3). Interestingly, two genera have both reported allergenic (AF) and potentially allergenic (PAF) fungal species – *Aspergillus* (8 AF, 4 PAF) and *Penicillium* (9 AF, 1 PAF). Of the 192 species reported from published literature, 158 were identified as allergenic, with the majority exhibiting Type I hypersensitivity reaction (Table 1). The genus *Alternaria* had the highest number of allergenic species, a total of 41 species (Table 2). This is followed by *Aspergillus* and *Penicillium*, with 8 and 9 identified species, respectively. The top ten genera with the greatest number of recorded allergenic fungi are shown in Table 2.

Table 1. The number of taxa per type of hypersensitivity reaction

	Type I	Type III	Multiple HR Types[a]	Total
Order	15	0	15	30
Genus	56	2	24	82
Species	122	3	33	158

[a]Multiple hypersensitivity reaction (HR) Types: Type I + III, Type I + IV, Type III + IV, Type I + III + IV

Table 2. The number of species recorded for the top 10 allergenic fungi

Genera	Number of Allergenic Fungal Species
Alternaria	41
Penicillium	9
Aspergillus	8
Curvularia	5
Fusarium	5
Calvatia	3
Cladosporium	3
Mucor	3
Stemphylium	3
Trichophyton	3

Table 3. List of fungal species with uncertain allergenicity and identities

Potentially allergenic fungi: species reported as allergenic in published literature but with no reported type of hypersensitivity reaction

Aspergillus flavipes	*Laetiporus* sp.	*Torula* sp.
A. giganteus	*Microsporum* sp.	*Verticillium* sp.
A. sydowii	*Monilinia* sp.	*Xylaria* sp.
A. ustus	*Paecilomyces* sp.	*Xylobolus* sp.
Botryotrichum sp.	*Penicillium solitum*	
Choanephora sp.	*Pithomyces* sp.	
Circinella sp.	*Plasmopara viticola*	
Cyathus sp.	*Pyrenochaeta* sp.	
Dacrymyces deliqeuscens	*Spegazzinia* sp.	
Geastrum saccatum	*Syncephalastrum racemosum*	

Allergenic fungi: species reported as allergens with the reported types of hypersensitivity reactions in published literature but with no species identity

Chaetomium sp.	Type I Hypersensitivity Reaction	Simon-Nobbe et al. (2007)
Claviceps sp.		Simon-Nobbe et al. (2007)
Cylindrocarpon sp.		Simon-Nobbe et al. (2007)
Erysiphe sp.		Kurup (2003)
Spondycladium sp.		Burrows et al. (1989)
Sporobolomyces sp.		Simon-Nobbe et al. (2007)
Tilletia sp.		Simon-Nobbe et al. (2007)
Tilletiopsis sp.		Simon-Nobbe et al. (2007)
Trichothecium sp.		Simon-Nobbe et al. (2007)
Urocystis sp.		Kurup (2003)

Lifestyle. Most of the allergenic fungi had varying life strategies from saprotrophic to biotrophic and endophytic and elicit a Type I hypersensitivity reaction (Table 4). However, our statistical analysis showed no relationship between the different lifestyles and the types of hypersensitivity reactions (Table 5, Figure 1), and the level of association was found to be negligible (Table 6).

Spore shape, texture, and color. The most prominent shape among the listed allergenic species was elliptical, followed by globose, ovate, fusiform, and quadrilateral (Table 4). Many species also exhibited variable shapes. Spore texture is generally smooth (92 species), albeit half of this number equivalent to 46 species had a rough texture. For spore color, the number of species is almost equally distributed between light- and dark-colored spores and a combination of both. A greater number of our listed species had Type I hypersensitivity reaction in relation to these spore traits, albeit we did not observe any strong relationship (Table 5, Figure 1), The Cramér's *V* coefficient also suggests a negligible to a weak association between the hypersensitivity reactions and these spore traits (Table 6).

Spore size, length, and width. Spore size, i.e., length plus width (Table 4), was mostly short and narrow (category IV, 93 species) followed by long and wide (Category I, 48 species). The observed χ^2 value, the significance probability, and the Cramér's *V* coefficient all showed no to a weak association between the type of hypersensitivity reaction elicited by the examined species and the overall spore size (Tables 5–6, Figure 1).

Table 4. Frequency of allergenic fungi per spore traits and types of hypersensitivity reaction

Characters	Categories	Type I	Type III	Multiple HR Types[a]	Total
Lifestyle	Biotrophic	4	0	0	4
	Endophytic	24	1	6	31
	Saprotrophic	28	1	10	39
	Varying Lifestyle	70	1	15	86
Spore Shape	Elliptical Shape	42	1	8	51
	Fusiform Shape	5	0	1	6
	Globose Shape	21	1	5	27
	Ovate Shape	5	1	1	7
	Quadrilateral Shape	1	0	1	2
	Variable Shape	59	0	16	75
Spore Texture	Smooth	69	2	21	92
	Rough	37	1	8	46
	Smooth to Rough	21	0	2	23
Spore Color	Dark Color	47	1	9	57
	Light Color	43	1	14	58
	Light to Dark Color	40	1	7	48
Spore Size (Length, Width)	I (Long, Wide)	44	0	4	48
	II (Long, Narrow)	12	0	2	14
	III (Short, Wide)	10	0	1	11
	IV (Short, Narrow)	65	3	25	93
Spore Length	Long (>20 µm)	56	0	6	62
	Short (≤20 µm)	75	3	26	104
Spore Width	Wide (>10 µm)	54	0	5	59
	Narrow (≤10 µm)	77	3	27	107
Type of Spore	Asexual Spore	97	2	24	123
	Sexual Spore	36	1	8	45
Presence of Appendages	Appendages Absent	127	3	31	163
	Appendages Present	4	0	1	5
Presence of Mycotoxins	Mycotoxins Absent	72	1	18	91
	Mycotoxins Present	61	2	14	77

[a]Multiple hypersensitivity reaction (HR) Types: Type I + III, Type I + IV, Type III + IV, Type I + III + IV

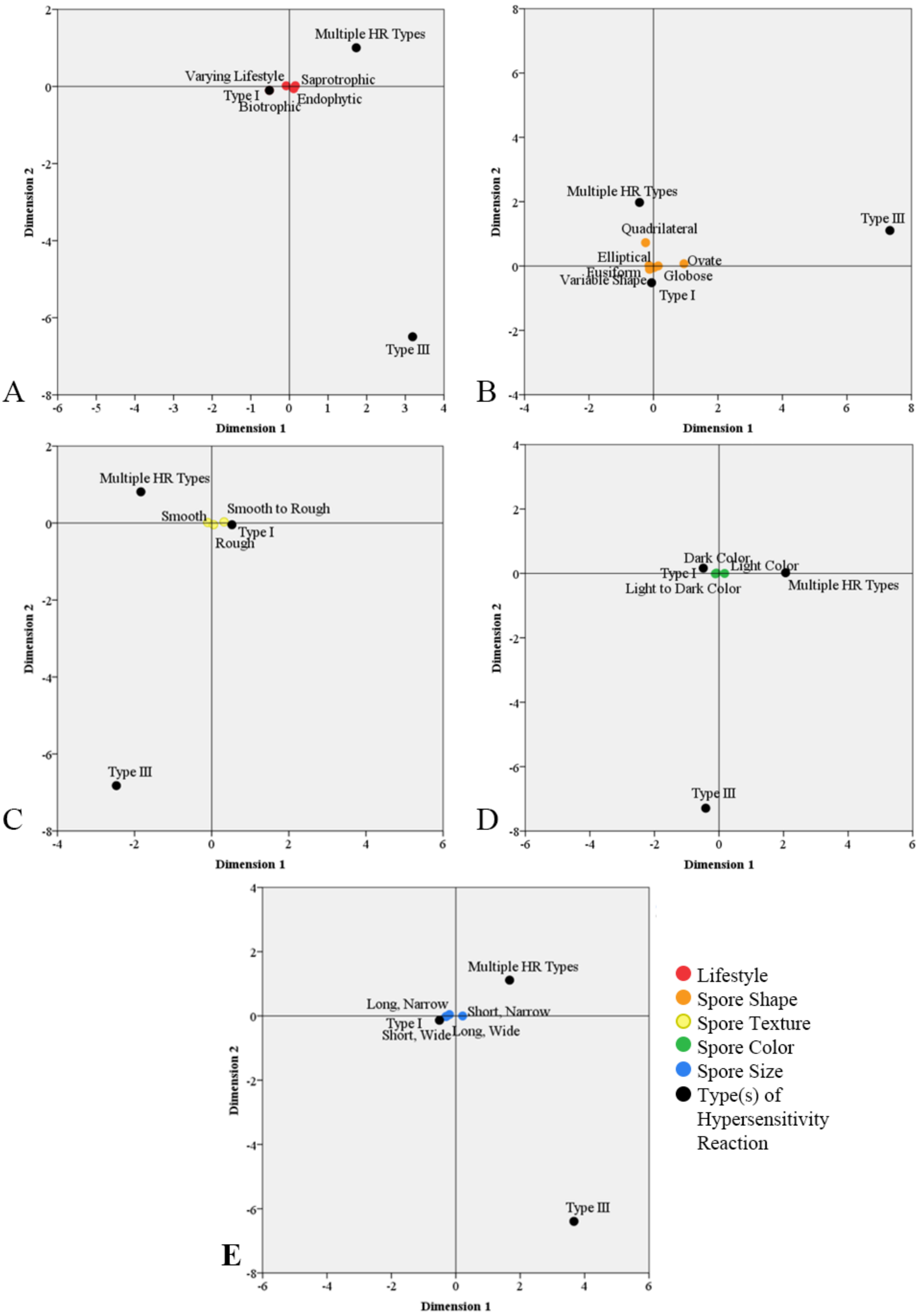

Figure 1. Association patterns between the types of hypersensitivity reaction and (A) lifestyle, (B) spore shape, (C) spore texture, (D) spore color, and (E) spore size of allergenic fungi

When spore size was categorized separately as length or width, the majority of the allergenic fungi exhibited a short length and a narrow width or diameter regardless of their hypersensitivity reaction (Table 4). Interestingly, while we did not observe any association between spore size and hypersensitivity reaction, there is a moderate association between spore length and the types of hypersensitivity reaction as elicited by the allergenic fungi (Tables 5–6). The same moderate association was also observed between spore width and HR types. Interestingly, the MCA plot showed that a short spore length is most likely associated with Type III and multiple types of hypersensitivity reactions while a long spore length is probably associated with Type I (Figure 2). Similarly, the MCA plot also showed that allergenic spores with a narrow width are likely associated with Type III and multiple types of hypersensitivity reactions while allergenic spores with a wide width may have an association with inducing a Type I hypersensitivity reaction.

***Other Characters*.** Most of the listed allergenic fungi have asexual spores. Their spores also had no appendages. The fungi also had no reported mycotoxins. And just like the other spore traits, these species' characteristics showed negligible association with the types of hypersensitivity reactions (Tables 4–6, Figure 2).

Table 5. χ^2 test of independence between the spore traits and types of hypersensitivity reaction

Characters	χ^2 **(Observed value)**	χ^2 **(Critical value)**	***P*** **(α = .05)**
Lifestyle	2.992	12.592	0.810
Spore Shape	10.147	18.307	0.428
Spore Texture	3.164	9.488	0.531
Spore Color	2.009	9.488	0.734
Spore Size	11.131	12.592	0.084
Spore Length	8.151	5.991	0.017
Spore Width	9.039	5.991	0.011
Type of Spores	0.123	5.991	0.940
Presence of Appendages	0.095	5.991	0.954
Presence of Mycotoxins	0.580	5.991	0.748

Table 6. Cramér's *V* coefficient for each of the spore traits of the allergenic fungi

Characters	**Cramér's *V***	**Interpretation of Association**[a]
Lifestyle	0.096	Negligible
Spore Shape	0.174	Weak
Spore Texture	0.099	Negligible
Spore Color	0.079	Negligible
Spore Size	0.183	Weak
Spore Length	0.222	Moderate
Spore Width	0.233	Moderate
Type of Spores	0.027	Negligible
Presence of Appendages	0.024	Negligible
Presence of Mycotoxins	0.059	Negligible

[a]Association Values: 0 – 0.100 (negligible), 0.101 – 0.200 (weak), 0.201 – 0.0400 (moderate), 0.401 – 0.600 (relatively strong), 0.601 – 0.800 (strong), 0.801 – 1.000 (very strong)

Spore traits of potentially allergenic fungi. These fungal species were reported in the published literature as allergenic species. However, there are no published reports on the types of hypersensitivity reactions elicited by these species, and hence, we categorized these fungi here as potentially allergenic. Given the association we observed between spore length and width and HR types, we predicted the probable types of hypersensitivity reactions that these potentially allergenic fungi will probably exhibit (Table 7). But it is important to note that laboratory tests must be conducted to verify these predictions.

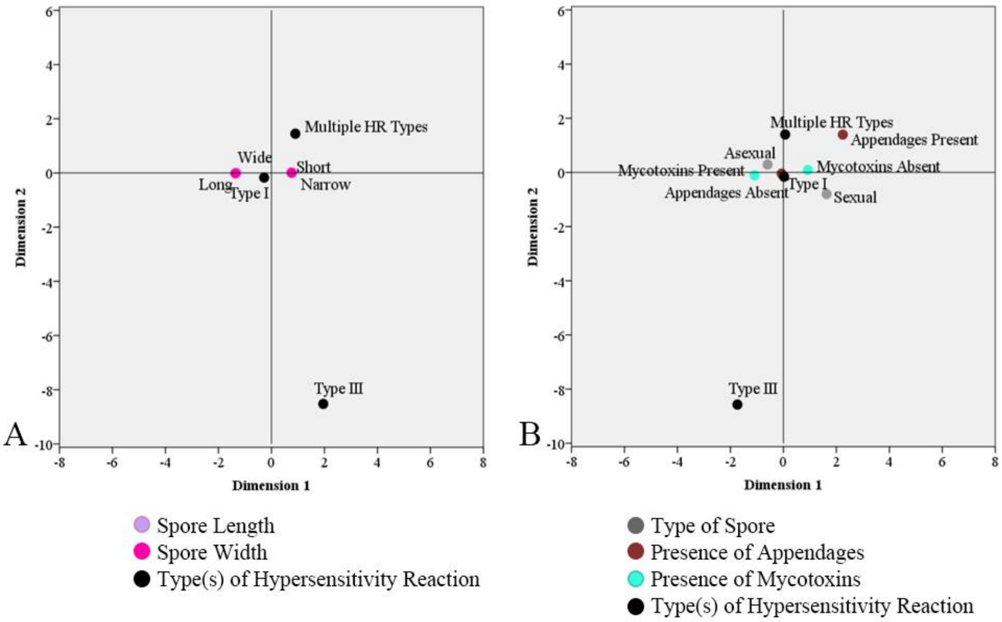

Figure 2. Association patterns between the types of hypersensitivity reaction and the (A) spore length and width, and (B) other traits of allergenic fungi

Table 7. Predicted types of hypersensitivity reactions for the listed potentially allergenic fungi based on the association patterns with spore length and width

Genus	Species	Length	Width	Predicted HR Type(s)
Aspergillus	*A. flavipes*	Short	Narrow	Type III or Multiple HR types
	A. giganteus	Short	Narrow	Type III or Multiple HR types
	A. sydowii	Short	Narrow	Type III or Multiple HR types
	A. ustus	Short	Narrow	Type III or Multiple HR types
Botryotrichum	*Botryotrichum* sp.	Short	Wide	Type I or Type III or Multiple HR types
Choanephora	*Choanephora* sp.	Short	Narrow	Type III or Multiple HR types
Circinella	*Circinella* sp.	Short	Narrow	Type III or Multiple HR types
Cyathus	*Cyathus* sp.	Short	Narrow	Type III or Multiple HR types
Dacrymyces	*D. deliqeuscens*	Short	Narrow	Type III or Multiple HR types
Geastrum	*G. saccatum*	Short	Narrow	Type III or Multiple HR types
Laetiporus	*Laetiporus* sp.	Short	Narrow	Type III or Multiple HR types
Microsporum	*Microsporum* sp.	Long	Wide	Type I
Monilinia	*Monilinia* sp.	Short	Wide	Type I or Type III or Multiple HR types
Paecilomyces	*Paecilomyces* sp.	Short	Narrow	Type III or Multiple HR types
Penicillium	*P. solitum*	Short	Narrow	Type III or Multiple HR types
Pithomyces	*Pithomyces* sp.	Long	Wide	Type I
Plasmopara	*P. viticola*	Short	Narrow	Type III or Multiple HR types
Pyrenochaeta	*Pyrenochaeta* sp.	Short	Narrow	Type III or Multiple HR types
Spegazzinia	*Spegazzinia* sp.	Short	Wide	Type I or Type III or Multiple HR types
Syncephalastrum	*S. racemosum*	Short	Narrow	Type III or Multiple HR types
Torula	*Torula* sp.	Short	Narrow	Type III or Multiple HR types
Verticillium	*Verticillium* sp.	Short	Narrow	Type III or Multiple HR types
Xylaria	*Xylaria* sp.	Short	Narrow	Type III or Multiple HR types
Xylobolus	*Xylobolus* sp.	Short	Narrow	Type III or Multiple HR types

Discussion

Pollen morphology has been associated with pollen allergenicity. The typical aerodynamic size of pollen, which ranges from 15–40 µm, allows for its deposition on the nasopharynx (D'Amato et al. 2007). There are also pieces of evidence that pollen could enter the lower respiratory tract, and hence, is implicated in asthma (Busse et al. 1972, D'Amato et al. 2007). Pollen that is associated with allergenicity lacks a distinguishable endexine layer in its pollen wall, which results in the leakage of allergenic proteins (Diethart et al. 2007). Like pollen, fungal spores have also been implicated in triggering hypersensitivity reactions among susceptible individuals. While fungal spores generally function in the species dispersal in different habitats and in species survival under unfavorable conditions (Dijksterhuis 2019), fungal spores when inhaled can cause hypersensitivity reactions as it contains immunoglobulin-specific antigens (Horner et al. 1995). In our systematic review, 158 fungal species were identified as capable of eliciting an allergic reaction. An additional 24 species were also listed as allergenic fungi but do not have any reported hypersensitivity reaction. This led us to look at spore traits and if any of its morphological properties are associated with a specific hypersensitivity reaction to predict the possible allergenicity of these potentially allergenic fungi.

Certain morphological traits may be factors to the fungal allergenicity. For example, spore shape can influence spore release, wherein an elliptical shape maximizes flight speed (Roper et al. 2008, Halbwachs and Bässler 2021), which in turn can be correlated with greater spore dispersal and persistence in the atmosphere. A globose spore shape can reduce the surface area to volume ratio, thereby facilitating better survival for the organism (Bergman and Casadevall 2010, Wang and Lin 2012, Calhim et al. 2018). Smooth spores or spores with simple ornamentation also allow for better wind dispersal (Diethart et al. 2007, Dawson et al. 2018) while the presence of spiny features on the spore surface can reduce contact, resulting in lower adherence (Whitehead et al. 2021). For spore color, pigments produced by fungi protect species from environmental stressors such as UV (Horner et al. 1995). Spore appendages can also function for attachment to a preferred host as observed with the spore dispersal mechanism of marine and freshwater fungi (Crous et al. 2012). However, unlike pollen morphology, these spore traits were not systematically tested for their association with hypersensitivity reactions.

In addition to spore traits, we also tested the correlation of lifestyles, type of spores, and mycotoxin production with allergenicity. Fungi are known to utilize various life strategies (i.e., biotrophic, endophytic, hemibiotrophic, necrotrophic, saprotrophic, or a combination of these) to facilitate adaptation in a changing environment (Girard et al. 2013, Muszewska et al. 2017). Asexual spores are produced in extensive numbers, ensuring that the organism can be quickly dispersed. Additionally, asexual spores serve as a resistance mechanism against harsh environmental conditions (Peberdy 1980). In contrast, the production of sexual spores increases genetic diversity, and when conditions are constantly fluctuating may select for the better-adapted strains (Sun and Heitman 2011). Mycotoxins also play a role in the virulence and pathogenicity of fungi, with the adverse effects being dependent on the level of exposure (Bush et al. 2006, Simon-Nobbe et al. 2007). Mycotoxins, e.g., gliotoxin and patulin, were considered aggravators of allergic asthma in murine models (Schütze et al. 2010, Kraft et al. 2021). Despite the potential of these spore traits (type, shape, color, texture, presence of appendages) and other species features (lifestyle, mycotoxin production) for dispersal, persistence, and survival of fungi, we did not find any strong association between these morphological features with any type of hypersensitivity reaction (Tables 5–6). However, we found a moderate association between spore size, particularly spore length and spore width, and types of hypersensitivity reaction (Tables 5–6, Figure 2).

A link between the size of the aeroallergens and the anatomical site to which these aeroallergens can be deposited is already established. For example, large spores are correlated with the upper respiratory airways, thereby causing allergic diseases in the nasopharyngeal area and the paranasal sinuses (D'Ovidio et al. 2021). In contrast, small spores were found to penetrate the lower respiratory tract and trigger allergic diseases of the bronchi and alveoli (Yamamoto et al. 2012, Dey et al. 2018). This link can be attributed to the spore deposition mechanisms in the respiratory tract, i.e., impaction and sedimentation (Deacon 2006).

The main mechanism, impaction, by which large spores are deposited, is observed during airflow fluctuation. This fluctuation causes the spores to diverge from their initial trajectories and impact the walls of the respiratory airways (Darquenne 2020). This only occurs in the upper respiratory airways since the structure of the nasal cavity increases air turbulence (Marieb and Hoehn 2018). Additionally, the size contributes to the momentum of large spores to impact and become trapped in the nasal mucosa (Deacon 2006). This elucidated why most large spores are associated with allergic sinusitis (Type I and/or Type III) and allergic rhinitis (Type I) (Simon-Nobbe et al. 2007, Ryan and Clark 2015). On the other hand, small size is insufficient for the spores to impact but enable the spores to reach the lower respiratory tract when the airflow is laminar (Deacon 2006). The primary mechanism for small spores is sedimentation, wherein the buoyancy of particles is reduced by gravitational forces (Darquenne 2020). In other words, the suspended small spores are acted upon by gravity, allowing them to finally settle on the surface or walls of the lower respiratory airways. Such phenomenon explains why small spores can induce multiple types of hypersensitivity reactions as manifested by allergic diseases such as hypersensitivity pneumonitis (Type III and Type IV), allergic bronchopulmonary mycosis (Type I, Type III, and Type IV), and the exacerbation of allergic asthma (Type I) (Mohr 2004, Simon-Nobbe et al. 2007, Agarwal and Gupta 2011, Shah and Panjabi 2014, Riario-Sforza and Marinou 2017, Pfavayi et al. 2020). Interestingly, some of the listed species with spores that are not entirely small in our review were implicated in allergic diseases affecting the lower respiratory tract. Sensitization to *Alternaria* and *Curvularia* were associated with severe allergic asthma, possibly due to the continuous release of allergens from the upper airways (Denning et al. 2014).

To confirm the types of hypersensitivity reactions induced by allergenic fungi, *in vitro* assays can be performed to assess the sensitizing potential of a spore. These assays include the radioallergosorbent test (RAST) which is specific to a type of immunoglobulin, the basophil activation test (BAT), the nephelometric detection of immunoglobulins or immune complexes, the radial immunodiffusion (RID), the lymphocyte transformation test (LTT), and the electro immunoassay (Virella et al. 1979, Whicher et al. 1984, Naisbitt et al. 2014, Foong et al. 2021). The enzyme-linked immunosorbent assay (ELISA) and Western blot analysis have also been used (Simon-Nobbe et al. 2007). To supplement the findings from these methods, *in vivo* allergy testing, specifically skin prick testing, is taken together with a history of the sensitized patient (Foong et al. 2021). We believe that spore size may also help with identifying potential allergenic fungi as an important preliminary step. In this study, we identified the possible type of hypersensitivity reactions that can be induced by potential allergenic fungi based on their spore length and spore width (Table 7). However, to confirm this, any of the *in vitro* assays must be conducted to validate our prediction.

In conclusion, following our systematic review, we found out that allergenic fungi with small spores are most likely able to trigger multiple types or a combination of hypersensitivity reactions. Fungal species with large spores, which can be associated with Type I hypersensitivity reaction, may be considered less potent to cause allergic diseases due to the limitations posed by the narrowing respiratory tract and deposition mechanisms. Spore morphological traits can offer insights into the allergenicity of fungi and can be utilized as a preliminary basis for hypersensitivity-related risk assessment of fungal species. Future studies may also focus on correlating spore morphological traits of other spore-bearing organisms such as myxomycetes (slime molds) to types of hypersensitivity reactions and/or specific allergic diseases.

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Statement on conflict of interest
The authors declare no conflict of interest.

Authors contribution
TEEDC conceptualized the research study. RRS, MCCG, SLRB, WTMC, and AMAM conducted the literature survey and data collection. RRS, MCCG, SLRB, WTMC, AMAM, NHAD, and TEEDC analyze the data and wrote the manuscript. The authors contributed equally to this work.

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