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Patterns and drivers of heat production in the plant genus *Amorphophallus*

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SUMMARY

Thermogenesis – the ability to generate metabolic heat – is much more common in animals than in plants, but it has been documented in several plant families, most prominently the Araceae. Metabolic heat is produced in floral organs during the flowering time (anthesis), with the hypothesised primary functions being to increase scent volatilisation for pollinator attraction, and/or to provide a heat reward for invertebrate pollinators. Despite in-depth studies on the thermogenesis of single species, no attempts have yet been made to examine plant thermogenesis across an entire clade. Here, we apply time-series clustering algorithms to 119 measurements of the full thermogenic patterns in inflorescences of 80 *Amorphophallus* species. We infer a new time-calibrated phylogeny of this genus and use phylogenetic comparative methods to investigate the evolutionary determinants of thermogenesis. We find striking phenotypic variation across the phylogeny, with heat production in multiple clades reaching up to 15°C, and in one case 21.7°C above ambient temperature. Our results show that the thermogenic capacity is phylogenetically conserved and is also associated with inflorescence thickness. Our study paves the way for further investigations of the eco-evolutionary benefits of thermogenesis in plants.

Keywords: *Amorphophallus*, pollinator attraction, reward, volatilisation, thermogenesis, phylogeny.

INTRODUCTION

Thermogenesis is the ability of an organism to raise its metabolism in order to elevate the temperature of its body or of particular tissues or organs and is characteristic of two major animal groups: mammals and birds. However, metabolic thermogenesis also occurs in plants, albeit rarely. With few exceptions, it is restricted to some gymnosperms and to early lineages of angiosperms. To date, it has been documented in 13 extant families of seed plants (Seymour, 2010). Of these, the best-investigated family, with the highest number of known thermogenic genera and species, is the Araceae, also known as the arum family (Grant et al., 2010; Ivancic et al., 2008; Mayo et al., 1997; Meeuse & Raskin, 1988; Seymour, 2010; Vogel, 1963, 1990).

Like in animals, plant metabolic thermogenesis is based on elevated mitochondrial respiration. Heat is produced through an intense increase in mitochondrial metabolism, during which carbohydrates or lipids are used as substrate by alternative oxidase (AOX) and/or by uncoupling proteins (UCPs) (Grant et al., 2010; Ito–Inaba, 2014; Miller et al., 2011; Onda et al., 2008; Seymour et al., 2015; Vogel, 1963, 1990; Wagner et al., 2008). Some thermogenic plant species are also thermoregulatory, which enables them to regulate the excess temperature to a certain extent

(Nagy et al., 1972; Seymour, 2004; Seymour et al., 1998; Seymour & Matthews, 2006; Seymour & Schultze–Motel, 1998).

Thermogenesis in the Araceae occurs during anthesis and is restricted to floral organs – more specifically, the male flowers and their derivatives, such as staminodes (Ivancic et al., 2008; Kakishima et al., 2011; Skubatz et al., 1990). Except for the genus *Taccarum* Brongn. ex Schott, the female flowers are not known to be thermogenic (Maia et al., 2013). The thermogenic patterns can vary strongly in timing, intensity and cycles (Kakishima et al., 2011). If an appendix (a sterile floral organ) is present, thermogenesis of the appendix and of the male flowers may occur either simultaneously or in an alternating pattern (e.g. Albre et al., 2003; Barabé et al., 2002; Chouteau et al., 2007; Gibernau & Barabé, 2000, 2002; Ivancic et al., 2004, 2005). The thermogenic phase usually lasts 2 days (Skubatz et al., 1990), but in extreme cases can last up to 30 days (Gibernau et al., 2010).

The functions of plant thermogenesis have long been debated and remain a contentious topic. The phenomenon has been associated with the prevention of freezing, spathe unfolding, anther dehiscence, carrion mimicry, infrared radiation, heat reward for pollinators, CO_2 release and the generation of heat for optimal pollen tube growth (Albre et al., 2003; Angioy et al., 2004; Dormer, 1960; Knutson, 1979; Korotkova & Barthlott, 2009; Patiño et al., 2002; Seymour, Yuka, et al., 2009; Vereecken & McNeil, 2010). Yet, the two most common hypotheses associate thermogenesis with (i) improved scent volatilisation during stigma receptivity and (ii) heat reward for insect pollinators. Such reward could be either direct – increasing the body temperature of the visiting insects – or indirect, by providing them with a heated floral chamber that could be used as a shelter, food place or mating site (e.g. Angioy et al., 2004; Bay, 1995; Meeuse & Raskin, 1988; Seymour & Gibernau, 2008; Seymour, Gibernau, & Itoh, 2003; Seymour, White, & Gibernau, 2003; Seymour, White, & Gibernau, 2009; Skubatz et al., 1990; van der Kooi et al., 2019).

Floral biology and thermogenesis in *Amorphophallus*

Amorphophallus Blume ex Decne. is one of the largest genera of the Araceae (Boyce & Croat, 2023). The genus consists of four subgenera: *Afrophallus* Hett. and Claudel, *Amorphophallus*, *Metandrium* Stapf and *Scutandrium* Hett. and Claudel (Claudel et al., 2017) and currently encompasses 237 species (Boyce & Croat, 2023). The genus *Amorphophallus* is widely distributed across the Old World tropics (Africa and Australasia) and is morphologically diverse (Claudel et al., 2017; Hetterscheid & Ittenbach, 1996; Pouchon et al., 2022) (Figure 1).

The inflorescence is protogynous (the female flowers become functional before the male flowers) and consists of a spadix surrounded by a spathe (Figure 2). The spathe is usually triangular or ovate and is more rarely funnel shaped (Hetterscheid & Ittenbach, 1996). It can be separated by a constriction, dividing the spathe into a floral chamber and an upper limb (Hetterscheid & Ittenbach, 1996). The spadix is subdivided into three main zones, with or without sterile delimitations in between (Hetterscheid & Ittenbach, 1996). The lowermost zone bears the female flowers and is adjacent to an intermediate zone that bears the male flowers (generally within the floral chamber), terminating with the final distal sterile zone – the appendix (above the floral chamber). The appendix is considered to be derived from fused staminodes (Mayo et al., 1997) and serves the biosynthesis and volatilisation of the scent compounds (Kite & Hetterscheid, 2017). Moreover, it serves as a landing or departing platform for the attracted insects (Gibernau et al., 2004). In *Amorphophallus*, the reported thermogenic zones are consistently both the male flower zone and the appendix (Korotkova & Barthlott, 2009; Lamprecht & Seymour, 2010; Shirasu et al., 2010; Skubatz et al., 1990).

Some *Amorphophallus* species develop large, dark inflorescences, accompanied by foul smells, and are referred to as *carrion* or *corpse flowers* (Chen et al., 2015; Lamprecht & Seymour, 2010), or more generally, oviposition-site mimics (Johnson & Schiestl, 2016; Jürgens et al., 2006, 2013; Jürgens & Shuttleworth, 2016). Oviposition-site mimicry refers to plant species that deceive and attract beetles and flies, which breed or feed on substrates such as carrion, dung, decaying matter or the like (Johnson & Schiestl, 2016; Jürgens et al., 2006, 2013; Kite et al., 1998; Kite & Hetterscheid, 1997, 2017; Moretto et al., 2019; Urru et al., 2011; Vereecken & McNeil, 2010). Additional features of floral oviposition-site mimicry include floral chambers, floral gigantism and thermogenesis (Johnson & Schiestl, 2016). Copro-necrophagous beetles are assumed to be the main pollinator group in *Amorphophallus* (Moretto et al., 2019). However, knowledge about the species identity of the pollinating invertebrates remains limited for most species in the genus (Claudel, 2021).

The key elements of oviposition-site mimicry are the scent compounds (Jürgens et al., 2013; Jürgens & Shuttleworth, 2016), which are very diverse in the genus and are usually unpleasant to a human nose, ranging from carrion, faeces, urine, dung, fish, sewerage, nauseating gases, rancid cheese to fermenting fruit and mushrooms (Claudel & Lev-Yadun, 2021; Kite & Hetterscheid, 1997, 2017). However, the species of two distantly related *Amorphophallus* clades are characterised by sweet fragrances or benzenoid compounds (Kite & Hetterscheid, 1997, 2017). Kite and Hetterscheid (1997, 2017) categorised 92 *Amorphophallus* species depending on the main emitted scent compounds and benzenoids represent one of seven scent categories *sensu* Kite and Hetterscheid (2017). One clade from the subgenus *Scutandrium* is characterised by the emission of 4-

Figure 1. Floral diversity within the four subgenera of *Amorphophallus*, exemplified here by some of the species analysed.
(a–c) *Amorphophallus antsingyensis*, *A. mossambiciensis* and *A. lewallei* from subgenus *Afrophallus*.
(d–g) *A. konjac*, *A. napalensis*, *A. longituberosus* and *A. fuscus* from subgenus *Scutandrium*.
(h–j) *A. myosuroides*, *A. prainii* and *A. bangkokensis* from subgenus *Amorphophallus*.
(k–m) *A. laoticus*, *A. symonianus* and *A. pilosus* from subgenus *Metandrium*. Scale bars: (a–m) = 10 cm. Photographs: Cyrille Claudel.

methoxyphenethyl alcohol, whereas the other clade from subgenus *Metandrium* is characterised by the emission of 2-phenylethanol derivatives (Kite & Hetterscheid, 1997, 2017). However, although sweetly scented, at least some of these compounds can be related to various stages of cadaveric decomposition (Claudel & Lev-Yadun, 2021).

To date, thermogenesis has been investigated in only 9 out of 237 *Amorphophallus* species (Barthlott et al., 2009; Handayani et al., 2020; Kakishima et al., 2011; Korotkova & Barthlott, 2009; Lamprecht et al., 2002; Lamprecht & Seymour, 2010; Prakash & Nayar, 2000; Shirasu et al., 2010; Skubatz et al., 1990; Teijsmann & Binnendijk, 1862; van der Pijl, 1937; Wagner et al., 1998). It has been proposed that thermogenesis in the appendix serves improved scent volatilisation (Barthlott et al., 2009; Handayani et al., 2020; Korotkova & Barthlott, 2009; Lamprecht & Seymour, 2010; Seymour, 2010), whereas thermogenesis in the male flower zone might also offer heat reward to pollinating insects (Handayani et al., 2020; Korotkova & Barthlott, 2009; Lamprecht et al., 2002; Seymour, 2010).

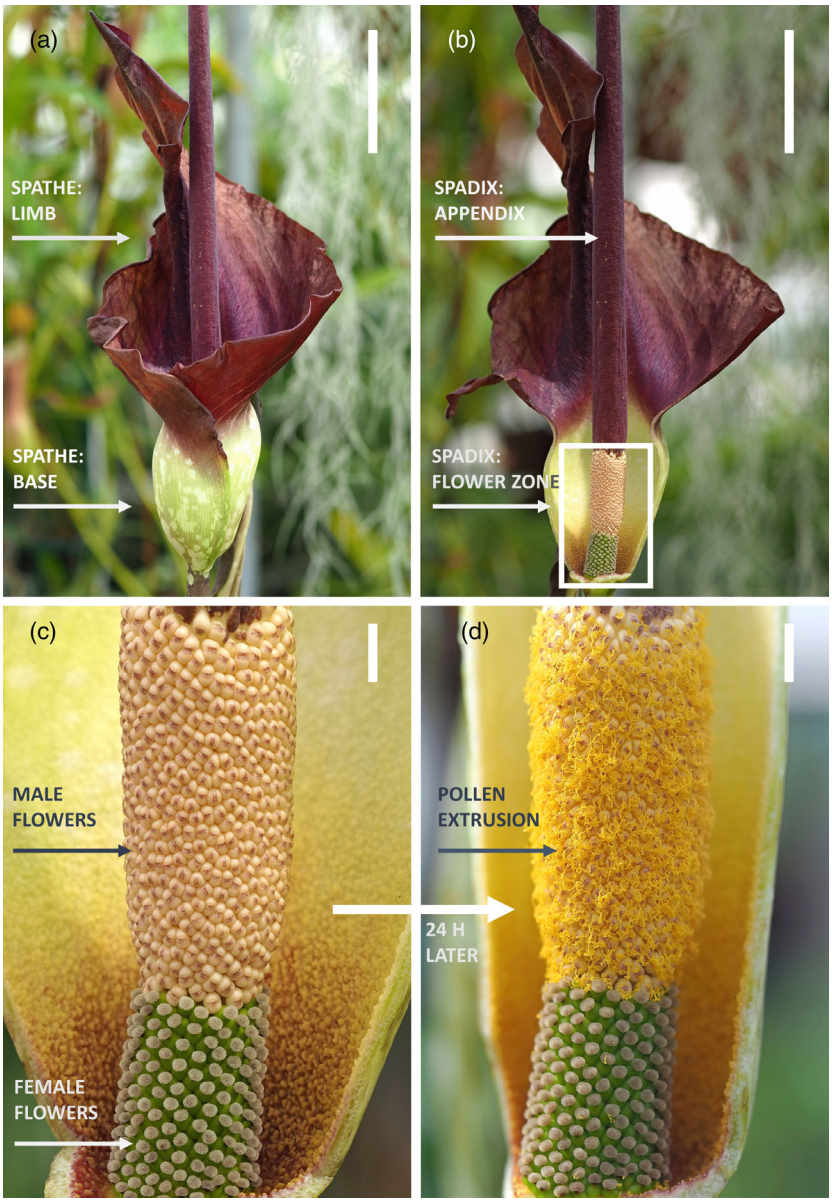

Figure 2. Inflorescences of *Amorphophallus* (here exemplified by *A. declinatus*).
(a) Inflorescence consists of a spadix and a spathe. The spadix has two parts: a limb and a base, the latter forming a floral chamber.
(b) The same inflorescence with the spathe base cut open to show the spadix composed of the appendix (above, outside the floral chamber) and the flower zone (below, within the floral chamber). The main thermogenic floral organs are the appendix and the male flower zone.
(c) First day of anthesis, close-up of the male flower zone (above) and the female flower zone (below).
(d) Second day of anthesis, pollen extrusion. Scale bars: a and b = 10 cm. c and d = 1 cm. Photographs: Cyrille Claudel.

However, to the best of our knowledge, these functions have never actually been tested. Moreover, although *A. krausei* Engl. (Wagner et al., 1998), *A. muelleri* (van der Pijl, 1937), *A. paeoniifolius* (Handayani et al., 2020; Lamprecht et al., 2002; Lamprecht & Seymour, 2010; Prakash & Nayar, 2000) and *A. titanum* (Barthlott et al., 2009; Korotkova & Barthlott, 2009; Lamprecht & Seymour, 2010; Shirasu et al., 2010) showed a significant temperature increase, only a moderate temperature increase could be observed in *A. bulbifer* (Roxb.) Blume, *A. forbesii* Engl. & Gehrm. (Skubatz et al., 1990) and *A. konjac* (Lamprecht & Seymour, 2010; Skubatz et al., 1990) whereas no temperature increase at all was observed in *A. gigas* (Kakishima et al., 2011; Teijsmann & Binnendijk, 1862) and *A. variabilis* Bl (van der Pijl, 1937).

Our literature compilation indicates that the knowledge of thermogenesis in *Amorphophallus* remains incomplete and biased towards a single species: *A. titanum*. The

lack of thorough data across most *Amorphophallus* species currently hinders our understanding of the patterns and potential role(s) of this fascinating phenomenon in the ecology and the evolution of the group.

Unravelling the evolution of thermogenesis in *Amorphophallus*

Beyond its functional role in particular species, the evolution of thermogenesis remains unexplored in plants. Although thermogenesis has evolved independently in several angiosperm families (Seymour, 2010; Thien et al., 2009), we do not know whether within *Amorphophallus* it originated once or multiple times independently. The physiological and morphological complexity associated with this phenomenon could potentially limit the lability of this trait and result in a high level of phylogenetic conservatism. Furthermore, it is not clear what could be the evolutionary fate of such a complex trait, considering the likely trade-off between the considerable costs of heat production (Lamprecht & Seymour, 2010) and its benefit for reproductive success. If this mechanism is too costly to maintain, a tendency towards the loss of thermogenic capacity across a thermogenic clade could be expected. Alternatively, if thermogenesis represents a cost-effective way to enhance pollination, the evolution of this trait might have positively impacted the diversification rate of heat-producing lineages or evolved multiple times independently within the genus. Besides a putative impact on diversification, given that thermogenesis occurs only in reproductive organs and may play a role in pollination, it could also have influenced the evolution of floral morphology.

The high species richness, large variation in inflorescence size and form, broad geographical distribution, well-documented scent production, reported occurrence of thermogenesis in multiple species and an available multi-locus phylogeny (Claudel et al., 2017), makes the genus *Amorphophallus* a suitable system for studying the evolution of plant thermogenesis. Here we apply quantitative measurements of the thermogenic activity of 80 species along with comparative phylogenetic methods in order to explore the relationship between thermogenesis and morphology in *Amorphophallus*. We ask, address and discuss the following specific questions:

Q1: Is thermogenic capacity an ancestral, phylogenetically conserved trait or did it evolve several times independently?

Q2: Has the evolution of thermogenesis triggered species diversification?

Q3: Is floral morphological evolution associated with the emergence of thermogenic capacity?

Q4: To what extent, can the thermogenic capacity be predicted from morphological traits?

RESULTS

Thermogenic activity

Graphs of the temperature measurements from all 80 species represented by 119 specimens are provided in Data S1, while thermal images from selected species are presented in Figure 3, highlighting key biological aspects of thermogenesis as well as some technical considerations related to thermogenic recordings. Additionally, time-lapse movies based on thermal images were generated for eight selected species representing the four subgenera, namely *A. albispathus*, *A. lewallei*, *A. paeoniifolius*, *A. prainii*, *A. schmidtiae* pattern 1, *A. schmidtiae* pattern 2, *A. tuberculatus*, *A. yunnanensis* (Data S2 embedded movie).

Our measurements show that thermogenesis in *Amorphophallus* is restricted to the male flowers, the staminodes and the appendix. However, the thermogenic pattern of the staminodes and the male zone are largely identical and therefore staminodes are not discussed further. Similarly, the female flowers are not discussed since they did not exhibit temperature increase, except for a few species that had a strong temperature increase in the adjacent male flower zone. In these cases, the observed temperature increase in the female flower zone is due to passive heat transfer.

The beginning of anthesis was usually marked by the beginning of the first thermogenic peak of the appendix, whereas in several species, the end of anthesis was indicated by a decrease in temperature of the male zone (e.g. Figure 4. *A. symonianus*). Cooling of the male zone after pollen extrusion can be attributed to evaporation through the open pores or slits of the anthers. As pollen release generally coincides with the end of the anthesis, this cooling effect is not likely to have impacted the temperature pattern before pollen release. This is supported by the fact that the temperature curve of the male flower zone does not drop below ambient temperature before or during anthesis in nearly all investigated species. There are some exceptions, such as *A. brachyphyllus* and *A. juliae*, which may require specific investigation in future studies.

To illustrate the wide range of thermogenic patterns observed in *Amorphophallus*, eight selected distinctly different temperature curves are shown in Figure 4. These include the species with the highest temperature increase (*A. longituberosus*), and the species with the longest thermogenic activity (*A. schmidtiae*), one species with several peaks of the appendix temperature (*A. fuscus*), one species with several thermogenic peaks exclusively in the male flower zone (*A. vogelianus*), one species displaying thermogenic activity in the appendix only (*A. symonianus*), one species (*A. yunnanensis*) with a strong thermogenic activity of the male zone occurring prior to thermogenesis of the appendix and scent release (personal observation, C.C.), one species (*A. lambii*) with cooling of the appendix,

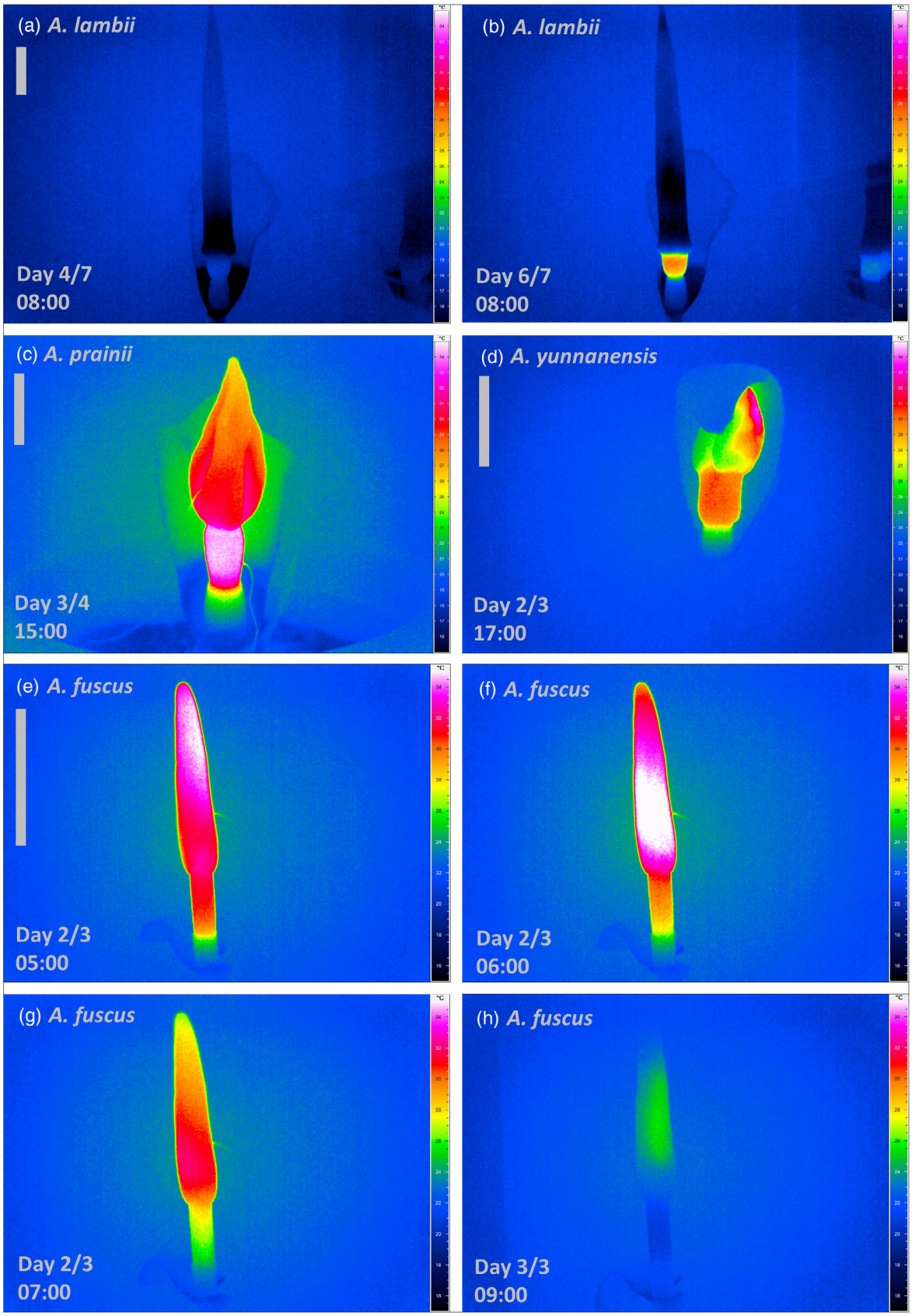

Figure 3. Thermal imaging observations in *Amorphophallus*.
(a) In *A. lambii*, the appendix and the spathe base cool down below ambient temperature. The cooling of the spathe base is remarkable, indicating evaporative cooling, possibly associated with scent emission by the spathe.
(b) Cooling of the spathe base and the appendix is not interrupted by thermogenesis of the male zone.
(c) Thermogenic peak of the male zone preceding the thermogenic peak of the heating-up appendix in *A. prainii*. The adjacent female zone below shows a temperature increase due to some passive heat transfer.
(d) *A. yunnanensis*. Although the overhanging spathe slightly blocks the view, strong temperature differences in the appendix can be observed, probably due to the irregularly folded appendix. In contrast, the male flower zone below is evenly warmed-up.
(e–h) Similarly, in *A. fuscus*, the appendix heats from bottom to top or from top to bottom rather than simultaneously all over. Different local temperature maxima are reached in the appendix, which illustrates the need for a consistent scheme for temperature sensor insertion.
(h) So far, *A. fuscus* is the only *Amorphophallus* species that ends anthesis with a temperature increase of the appendix. Scale bars: a–h = 10 cm. Day numbers are relative to the total duration of anthesis. Photographs: Cyrille Claudel.

but with a strong thermogenic activity of the male zone. Finally, one species (*A. lewallei*) with a distinct biphasic pattern, starting with a peak of the appendix temperature on the first day of anthesis, followed by a peak of the temperature of the male flower zone on the second day.

The full thermogenic sequence of most species (74/80) lasted approximately 48 h or slightly longer. However, we found several exceptions, such as *A. borneensis*, *A. consimilis*, *A. dracontioides* and *A. lambii* (Figure 4), where anthesis and thermogenesis lasted for about 5–6 days, and *A. schmidtiae* (Figure 4), an extreme case in which the thermogenic activity lasted up to 3 weeks.

In total, 20 species (25%) did not exceed a temperature increase of 1.5°C in the appendix and in the male flowers and we consider these species as 'non-thermogenic'. There were eight species (10%) that we consider 'weakly thermogenic' because they did not exceed ambient temperature by more than 2°C both in the appendix and male zone. Nearly half of the species (36/80 or 45%) exhibited a temperature increase of between 2°C and 10°C in at least one part of the inflorescence and we classify them as 'thermogenic species'. Sixteen species (20%) exceeded ambient temperature by 10°C or more in at least one part of the inflorescence, and these are referred to as 'strongly thermogenic' in the following discussion. Repeated analyses of plants clonally propagated through tuber multiplication showed that the thermogenic pattern is usually similar and reproducible within clones (ramets) of the same genet. Examples included *A. interruptus*, *A. lewallei*, *A. myosuroides*, *A. napalensis*, *A. prainii*, *A. tenuispadix* and *A. thaiensis*. One notable exception was found in *A. schmidtiae*, where the clones exhibited two different thermogenic types. In the first type, thermogenic activity lasted several weeks, even though scent emission was only noticeable by a human nose on the first day of anthesis. In contrast, thermogenesis lasted 2 days in the second type, similar to many other *Amorphophallus* species.

Thermogenic patterns were usually similar between different individuals of a given species, as observed in *A. curvistylis*, *A. myosuroides*, *A. napalensis*, *A. prainii* and *A. tuberculatus*. However, a certain amount of intraspecific variation was found in some of the species. For example, the beginning of anthesis differed between *A. albispathus* HBG 2014-G-37, and *A. albispathus* HBG 2014-G-39 (Data S1). Likewise, the beginning of anthesis and the number of appendix peaks slightly differed between the two documented accessions of *A. albus* (Data S1). These results indicate that thermogenic patterns are largely reproducible under similar conditions, although further investigations may provide a more detailed understanding of intraspecific variation. We cannot exclude that several variables, such as ambient temperature, air humidity, airflow and plant size might influence the thermogenic activity to a minor extent. Consequently, the temperature peaks might be higher under higher ambient temperature and relative humidity, unless the inflorescence is thermoregulated – a factor not directly investigated in our experiments.

Time-series clustering

The cluster dendrogram resulting from the multivariate time-series analyses of appendix and male zone temperature series show that most thermogenic species cluster together, separated from most of the non-thermogenic or weakly thermogenic species (Figure 5). However, our analyses also show that, because thermogenesis is not equal in the different parts of the inflorescence, some thermogenic species fall in fact into the mostly non-thermogenic cluster. For example, in *A. symonianus* and *A. scutatus*, thermogenic activity is high in the male zone but weak in the appendix and they both cluster with species that have low activity in both parts of the inflorescence (Figure 5). However, both *A. coudercii* and *A. lambii* have a high thermogenic activity restricted to the appendix, but while *A. coudercii* clusters with other species that have a high thermogenic activity in the appendix, *A. lambii* stands out among a group of weakly thermogenic species, which may be explained by the unusual cooling of the appendix below room temperature observed in that species. The two dendrograms from the univariate time clustering analyses are broadly similar, both displaying two main clusters, one of species with medium to high-temperature increase and a second of weakly or non-thermogenic species (Figure S1).

In the analysis with intraspecific sampling, replicate individuals cluster together in *A. atroviridis*, *A. bulbifer*, *A.*

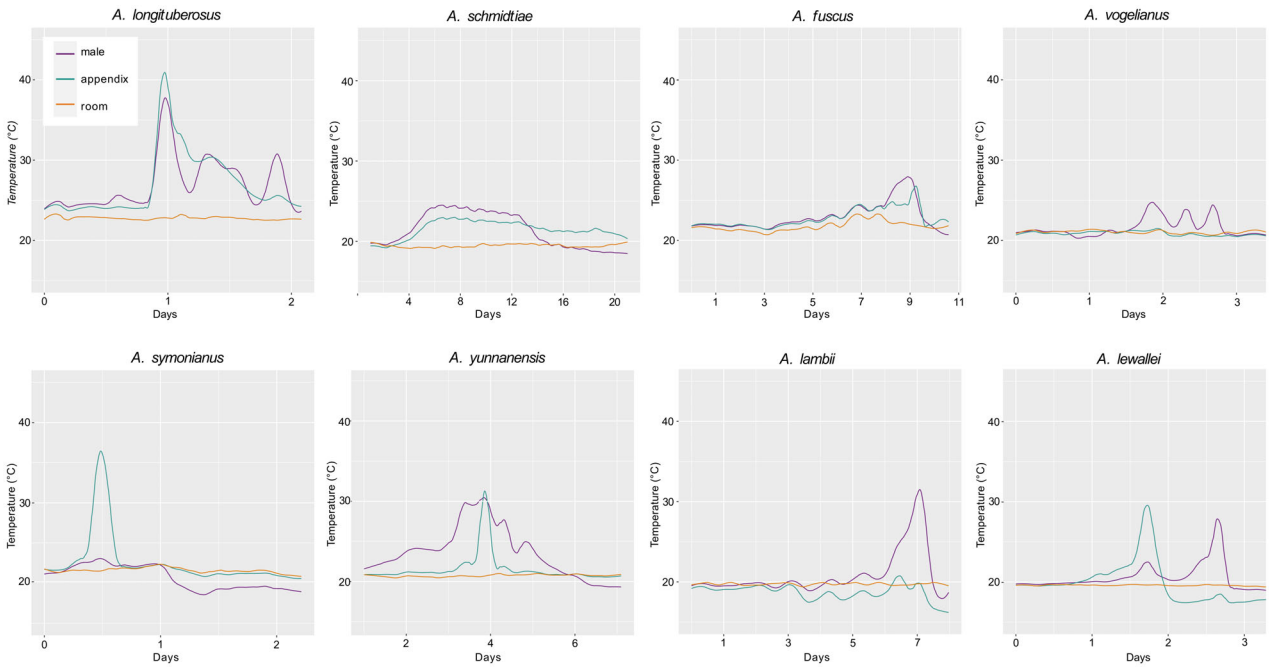

Figure 4. Time series of temperature in the appendix and the male flowers zone measured in eight different species of *Amorphophallus*. For display purpose, raw measurements have been smoothed with a loess function.

interruptus, *A. lewallei*, *A. myosuroides*, *A. opertus* and five out of the seven individuals of *A. schmidtiae* (Data S3). For the remaining 14 species with multiple samples, individuals did not form an exclusive cluster, although they often belonged to the same broader cluster.

Time-calibrated phylogeny

The MCMC analyses in BEAST (Bouckaert et al., 2014) converged and all parameters have effective sample size values above 300. The topology of the inferred phylogenetic tree is very similar to the previously recovered one in Claudel et al. (2017), with an improved resolution despite not being fully resolved (Figure 6; Figure S2), with 63% of the nodes have a posterior probability above 0.8 and 55% above 0.9. Hence, although the monophyly of each of the four subgenera is well supported, their relationship with one another remains inconclusive, a result encountered in most previous studies (Claudel et al., 2017; Sedayu et al., 2010) but resolved and discussed in Pouchon et al. (2022).

Models of diversification

The most likely model of diversification selected in the model comparison analysis is a birth-death model with all parameters (λ, μ and net diversification rate) decreasing linearly through time (Figure 6). The pulled speciation rate also decreases through time, from 0.33 at the root, to 0.069 at the tips, with a sharp decrease before 25 MY and a plateau between 25 MY and 10 MY (Figure 6), suggesting that the estimated speciation rate decrease is robust to identifiability issues. In the HiSSE analysis, the best-fit model is the character-independent model (ΔAIC = 2.61, Table S1), indicating that the evolution of thermogenesis is not associated with significant changes in diversification rates across lineages.

Evolution of thermogenesis

Through ancestral state estimation performed on thermogenesis treated either as a binary or continuous trait, thermogenic capacity was inferred to be of a single origin in *Amorphophallus*, present at the origin of the group and subsequently lost in several species belonging to different clades (Figure 7).

Display of peak temperature of the inflorescence during anthesis at the tips of the phylogeny shows that the thermogenic capacity is not a randomly distributed trait: despite missing data, some clades clearly appear to be made up of mostly weakly thermogenic species, and others of mostly of strongly thermogenic species. For example, the subgenus *Amorphophallus* comprises mostly of weakly or non-thermogenic species, such as the Philippine species *A. declinatus*, the Brachyphyllus clade, the Pusillus clade and the Pulchellus clade (Figure 6). In contrast, the subgenus *Metandrium* contains both a clade with weakly or non-thermogenic species (Pygmaeus clade) and a clade of strongly thermogenic species, that is the

phenylethanol scents clade (Figure 6). Finally, the most strongly thermogenic species of the African subgenus *Afrophallus* (*A. mossambicensis* and *A. lewallei*) are sister species, but no reliable trend can be inferred in this clade due to the low number of sampled species. This visual pattern is confirmed by Pagel's lambda estimates (λ = 0.552 for the appendix' peak temperature, and λ = 0.568 for the male zone's peak temperature), which indicate a significant amount of phylogenetic signal, although less than expected under Brownian motion evolution (see Figure 8).

Association between thermogenesis and morphological evolution

The overall amount of phylogenetic signal across the morphological dataset is low, as most values of lambda on the phylogeny are below 0.5, except for mean pollen size, spadix length, the ratios of appendix length/spadix length, peduncle length/peduncle diameter (Figure S3). On the cluster dendrogram, lambda values are generally close to zero, except for three traits which have a high lambda on the cluster dendrogram: male zone radius (λ_{dendro} = 0.9620), peduncle diameter (λ_{dendro} = 0.8639), spathe length (0.6871) and width (λ_{dendro} = 0.8214) (Table S2).

Our multiple regression model showed a significant positive correlation between the peak temperature in the appendix and the radius of the male zone (Figure 9; Table S3). The same association was recovered for the peak temperature of the male zone but was not significant. We also found a significant but small negative correlation between the height of the male zone and the peak temperature of the appendix (Figure 9; Table S3).

DISCUSSION

Our study provides an unprecedentedly detailed and consistent recording of thermogenesis for 80 plant species belonging to the Araceae genus *Amorphophallus*. We find substantial evidence of widespread thermogenic activity in the genus, a phenomenon that we report for species in all continents where this plant group occurs, and which is produced by species with vastly different morphologies – from huge to small inflorescences.

Relevance of time-series clustering to the study of thermogenesis

Thermogenesis is a highly complex and dynamic biological phenomenon that challenges attempts to classify thermogenic types. The approach developed here aimed at tackling this complexity in a more statistically and biologically realistic way, by taking into account the full temporal trajectories of temperatures. Our results show that applying time-series clustering to temperature measurements in the inflorescence of thermogenic species is a coherent and powerful approach, which enables a more biologically realistic classification of thermogenic patterns. Indeed, instead of focusing on a single aspect of thermogenesis, that is, the peak of temperature elevation, clustering of the several day long full time series takes into account the full thermogenic pattern of each species. This approach, therefore, integrates the natural complex variation observed in this trait which includes the potentially differential temperature elevation of the different parts of the inflorescence, as well as either their synchronicity or temporal separation and the overall trend in temperature increase throughout anthesis (e.g. linear increase, single or multiple peaks). The time-series clusters identified are therefore different from the groups we would have observed had we classified species based solely on the peak of their temperature elevation (Figure 5). For example, *A. symonianus* and *A. scutatus* are two strongly thermogenic species clusters within a group of species that are mostly weakly thermogenic, yet all of these species share a common characteristic: a net cooling of the male flowers part after pollen release once anthesis has ended (Data S1). Likewise, *A. lambii*, a species with a strong temperature peak in the male zone clusters with species that exhibit only such a small peak, yet all of them share a simultaneous cooling of the appendix. In summary, our analysis of temperature curves revealed that thermogenesis displays great biological variation across *Amorphophallus*, in terms of duration, location in the inflorescence, intensity and shape, a strong indication of the evolutionary flexibility of this trait.

A phylogenetic perspective on thermogenesis

Q1: Is thermogenic capacity an ancestral, phylogenetically conserved trait or did it evolve several times independently?

Ancestral reconstructions of peak temperature at anthesis suggest that the presence of thermogenesis is an ancestral character in *Amorphophallus* and that this capacity has been lost several times in non-sister clades during the long evolutionary history of the group. Despite considerable variation in thermogenic patterns, temperature increase during anthesis still exhibits some degree of phylogenetic conservatism as indicated by Pagel's lambda intermediate values and this is reflected in some clades being made up of mostly strongly thermogenic, or mostly weakly thermogenic species.

Considering that we had temperature data for only half (80/157) of the species included in the *Amorphophallus* phylogeny, the precise sequence of trait shifts (i.e. origin and loss of thermogenic capacity) remains to be confirmed with a more comprehensive species dataset. However, we believe that future analyses will not undermine our main result, which is that the presence of thermogenesis is an ancestral character in *Amorphophallus*. Indeed, our sampling was sufficient to show that thermogenic activity is present across the whole genus (Figure 5)

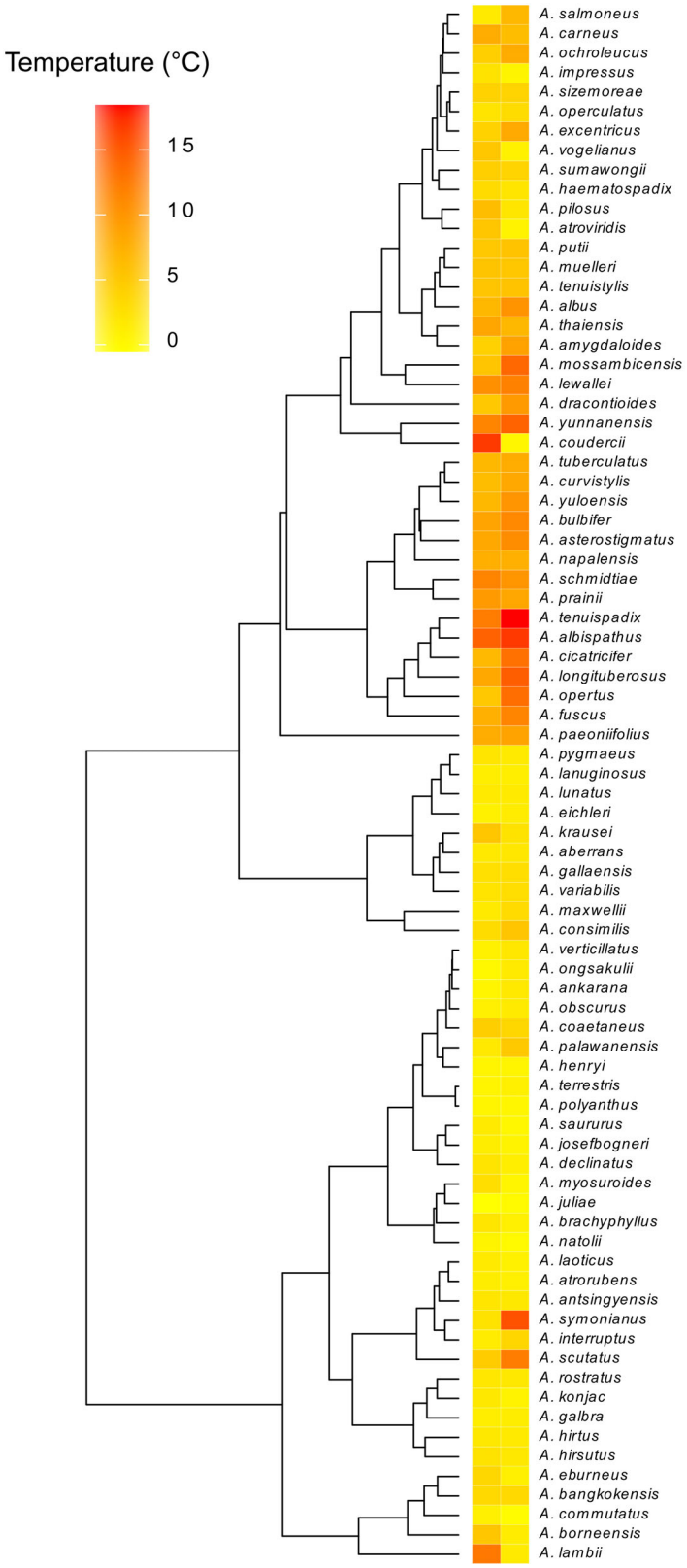

Figure 5. Cluster dendrograms based on multivariate time-series clustering of temperature series obtained from measuring temperature increase above room temperature during anthesis in the male zone and appendix. Coloured squares at the tips represent species' peak temperature in °C.

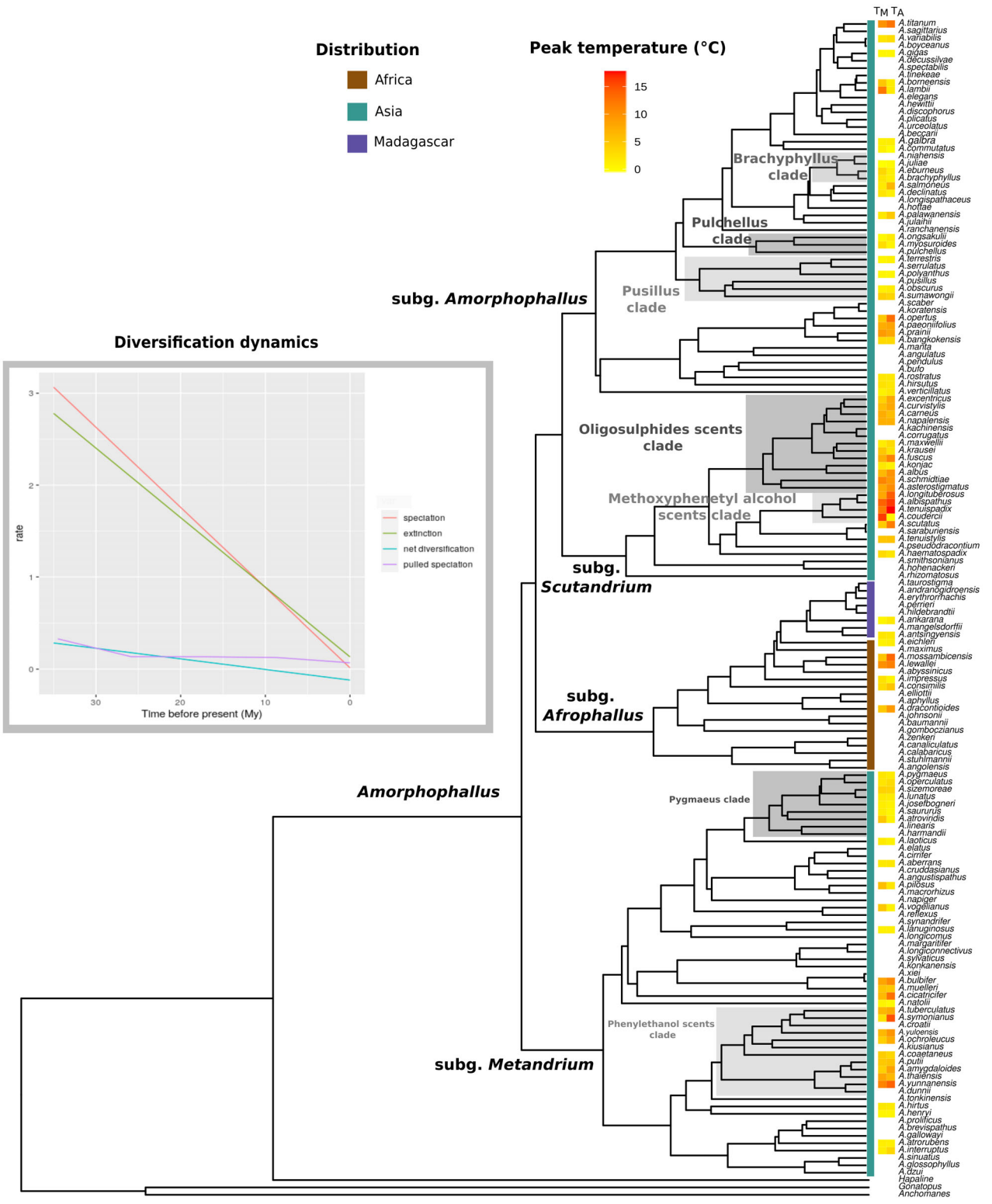

Figure 6. Time-calibrated phylogeny of *Amorphophallus*. Coloured dots at terminals indicate species' broad geographical distribution; followed by coloured squares which indicate the thermogenic activity measured in this study, by showing peak temperature during anthesis in the male zone (left) and the appendix (right) of the inflorescence. Names of subgenera are provided at notes and clades mentioned in the text are delimited with colour-coded boxes. The inset box on the left shows the speciation, extinction and net diversification rate through time inferred under a time variable birth-death model and the pulled speciation rate. Time in millions of years from the present.

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and that only 15% of the analysed species did not exhibit significant temperature increase, which indirectly supports the scenario of a single origin early in the evolution of the genus, with subsequent losses, reductions or increases. The exact evolutionary advantage of this trait and its genomic underpinning are beyond the scope of this study and remain to be investigated. A possible scenario could be that once it evolved, the genetic assemblage necessary for a thermogenic capacity was retained throughout the evolutionary history of *Amorphophallus*, and that the observed variation in thermogenic patterns (e.g. duration and intensity of temperature elevation, differences in a location within the inflorescence) is controlled by regulatory mechanisms at the level of gene expression. Under this scenario, genes necessary for thermogenesis may still be present, but not fully expressed, even in non-thermogenic species. Comparative transcriptomic studies of non-thermogenic, weakly thermogenic and strongly thermogenic *Amorphophallus* species would allow us to test this hypothesis and shed light on the genomic underpinning of this complex physiological trait.

Q2: Has the evolution of thermogenesis triggered species diversification?

Results from the two analyses of diversification (Table S1) suggested that the diversification rate of *Amorphophallus* decreased over time. Furthermore, the rejection of a trait-dependent model in favour of a null, trait-independent one, suggests that the evolution of thermogenesis did not significantly impact the diversification rate within the group. This is coherent with our finding that thermogenesis was present at the origin of the group and is not restricted to a certain clade. Hence, thermogenesis, at least within *Amorphophallus*, seems to be decoupled from the rate of diversification, although additional data would be necessary to further test this hypothesis beyond the species included in our analyses. A macroevolutionary analysis across the entire Araceae family could confirm that the evolution of thermogenesis is truly decoupled from the rate of diversification or alternatively show that an effect on species diversification can only be detected over a larger evolutionary timeframe.

Q3: Is morphological evolution primarily linked to thermogenesis or to shared ancestry?

Given the probable occurrence of thermogenesis early in the evolutionary history of *Amorphophallus* and its putatively important ecological role in pollinator attraction, we asked whether this trait has influenced the evolution of inflorescence morphology. If the constraint played by thermogenic capacity on morphological traits were stronger than the effect of shared ancestry, we would expect Pagel's lambda values for morphological traits to be higher on the dendrograms of peak temperature than on the phylogeny. Our findings revealed that three traits (male zone radius, peduncle diameter and spathe width) had very high lambda values on the temperature dendrogram. This suggests that the evolution of inflorescence thickness or width is tightly linked to the thermogenic pattern. Interestingly, the other morphological traits, which appear to have evolved independently of thermogenic capacity, were only weakly conserved phylogenetically. This suggests that the evolution of most floral traits in *Amorphophallus* is highly labile or linked to other factors, such as environmental variables that were not included in our analyses.

Q4: To what extent can individual morphological traits predict the strength of the thermogenic capacity?

The fact that weakly or non-thermogenic species include the smallest species in the genus, bearing inflorescences not exceeding a few centimetres in length (Claudel et al., 2017), hints that thermogenesis may be linked with the evolution of large inflorescences. However, the opposite is also true: some species with the highest recorded temperature elevations, such as *A. albispathus*, are among the smallest species. Finally, some of the largest species, for instance, *A. gigas*, display no temperature elevation at all (Kakishima et al., 2011) and see Claudel et al. (2019) for alternative hypotheses to explain floral gigantism in *Amorphophallus*.

Beyond anecdotal evidence, we formally tested for a possible correlation between overall inflorescence morphology and thermogenic activity in *Amorphophallus*. Our results reject the simplistic view that larger species are more likely to be thermogenic. Instead, we found that temperature increase is mostly associated with the width of the inflorescence as indicated by the positive association between appendix peak temperature and width of the male zone. Similar but non-significant positive associations were found between male zone peak temperature and width, and between the width of the appendix and peak temperature in both the appendix and male zone. We also found a significant, although weak, correlation between the appendix and male zone's height with peak temperature in the male zone and in the appendix, respectively. These results suggest a tendency for thermogenesis to be stronger in species which are shorter but thicker. These traits are characteristic of an overall robustness of the inflorescence, a typical adaptation to beetle pollination (Bernhardt, 2000; Kevan & Baker, 1983). Thick and warm inflorescences may play a role in pollinator attraction or provide a warm shelter for visiting insects, but data on visiting insects and pollinators are too limited to confidently test these hypotheses (Claudel, 2021). The overall weak relationship we found between thermogenic activity and inflorescence morphology suggests that there is no emblematic thermogenic inflorescence and that floral traits cannot be used as a proxy for temperature measurements

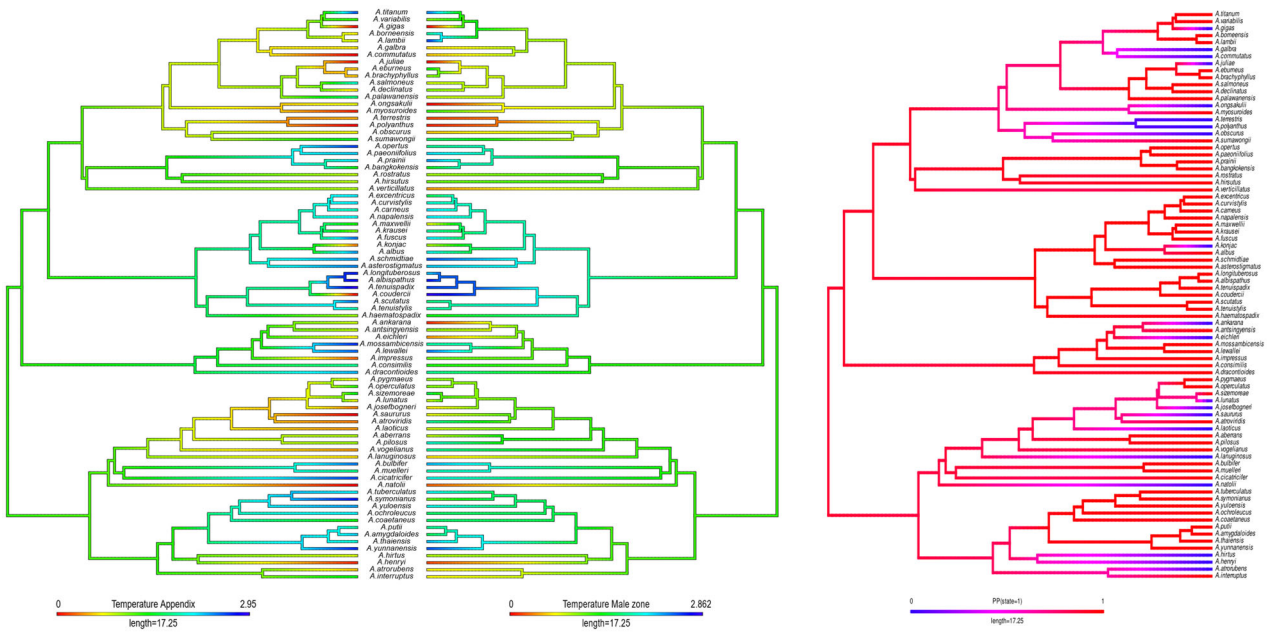

Figure 7. Ancestral state reconstruction of thermogenic activity treated as a continuous character (log peak temperature of the appendix, left; and of the male zone, middle) and as a binary trait (right).

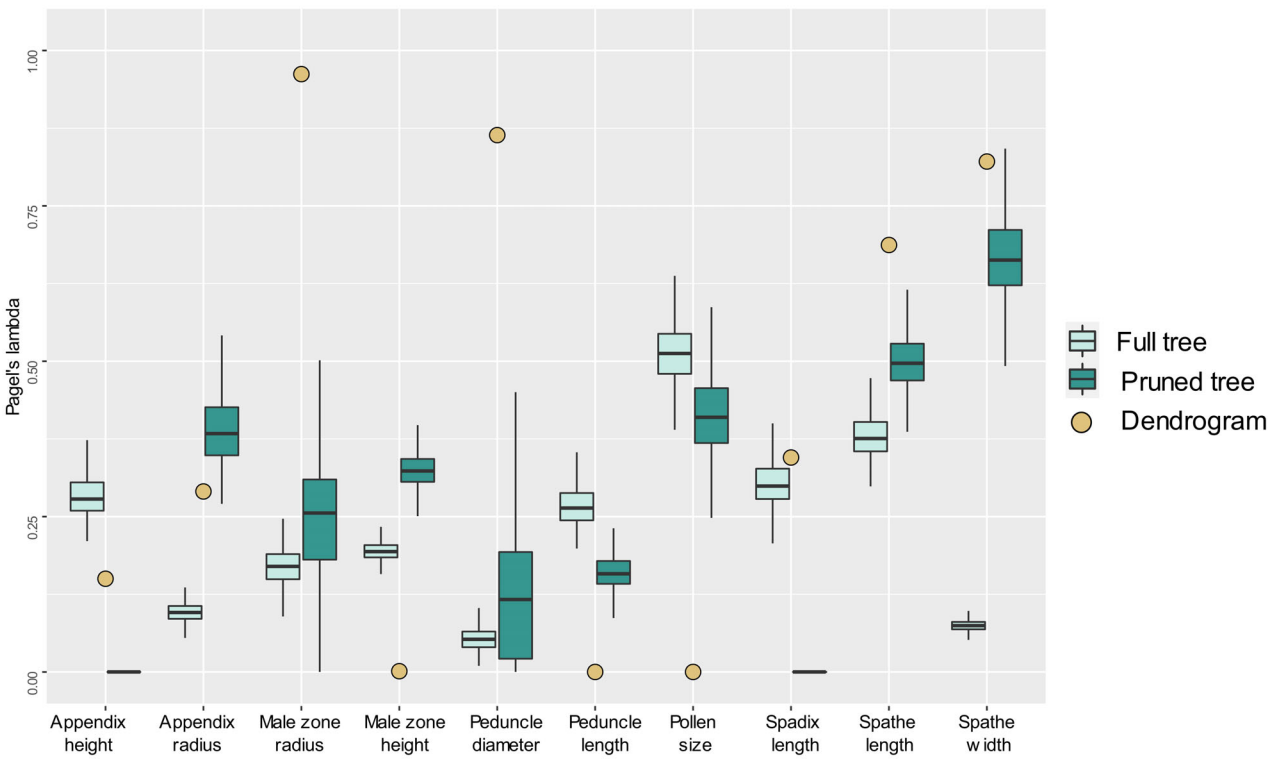

Figure 8. Pagel's lambda values estimated for morphological traits on (1) cluster dendrogram (yellow dots), and (2) a set of 100 trees representing the whole phylogeny (light green), or a pruned phylogeny with the same number of species as cluster dendrogram (dark green).

to reliably predict whether a species is thermogenic. Although we tried to include as many variables as possible in our analyses, we cannot exclude that thermogenic activity may actually have a stronger correlation with other morphological traits such as the number and size of excreting pores.

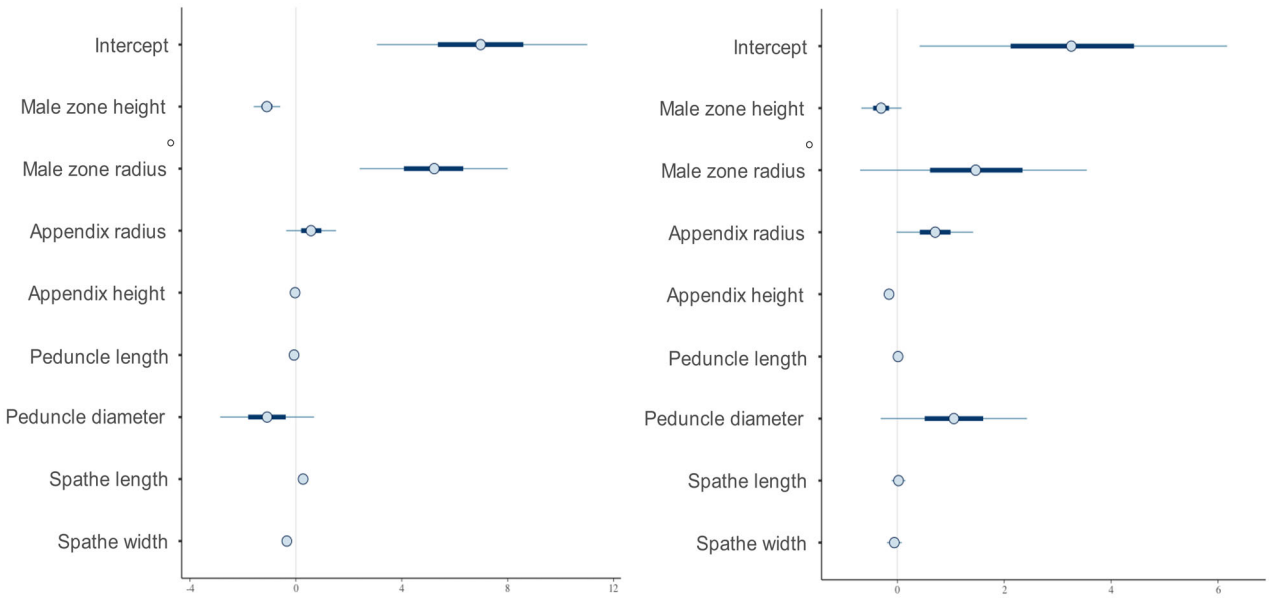

Figure 9. Result of multiple regression between morphological variables and peak temperature in the appendix (left) and in the male zone (right).

Deciphering thermogenesis: future directions

Our findings shed new light on the biology and evolution of metabolic thermogenesis. Despite these advances, the exact function/s of temperature elevation during anthesis remains elusive. For example, the correlation we found between thermogenesis and floral morphology typical of beetle pollination does not shed light on the putative function of temperature elevation in scent emission and pollinator attraction. Thermogenesis could potentially contribute to CO_2 release and thus to the attraction of invertebrate pollinators (Patiño et al., 2002). Indeed, CO_2 detection is widespread in insects (Jones, 2013), which are known to rely on CO_2 gradients for locating suitable food sources (Jones, 2013; Vereecken & McNeil, 2010).

Interestingly, two species groups from the subgenera *Scutandrium* and *Metandrium*, here named in reference to their emitted major scent compounds, the methoxy phenethyl alcohol scents clade and the phenylethanol scents clade (Figure 5), produce sweet, fruity or almond odours based on aromatic hydrocarbons (Kite & Hetterscheid, 2017). These clades comprise mostly thermogenic or strongly thermogenic species, in particular *A. albispathus*, *A. longituberosus* and *A. tenuispadix*, all of which can exceed 15°C above room temperature. One of the greatest temperature elevations recorded in a plant species so far, for example *A. longituberosus* (21.7°C above ambient temperature, Figure 5), is only matched by two other species within the Aroideae (Ivancic et al., 2005; Nagy et al., 1972). However, the putative role of thermogenesis in the volatility of these scent compounds has not been formally tested.

Taken together, the available evidence suggests that rather than playing a fixed and universal role, the function of thermogenesis is likely to vary with the species' ecology, depending for instance on whether a species emits specialised scent compounds or occurs in cooler climates (subtropical regions or high elevation in the tropics). We hypothesise that if such links exist, they are largely loose and non-deterministic, similar to what we found for morphological traits.

A final aspect that deserves closer attention in future studies is the occasional uncoupling of temperature elevation in the appendix and the male flowers zone, in species that heat up only one of the floral organs. A deeper study of such patterns might reveal that the heating of different parts of the inflorescence plays a different role. Thus, the contribution of thermogenesis to pollination success needs to be investigated experimentally for both floral organs independently.

Methodological considerations

Temperature elevation in tissues might not fully quantify total thermogenesis, due to potential heat loss through evaporation or other physical constraints (Gibernau et al., 2005; Lamprecht & Seymour, 2010; Seymour, 2010; Seymour & Schultze–Motel, 1999). Therefore, to quantify thermogenesis, the use of respirometry instead of temperature measurements has been emphasised (Seymour, 2010). It has been argued that (evaporative) heat loss due to the high surface area of the appendix can significantly lower the temperature despite high respiration rates (Lamprecht & Seymour, 2010; Seymour, 2010; Seymour & Schultze–

Motel, 1999). Moreover, it has been argued that evaporation could lead to water droplet formation on the appendix of *A. konjac* (Lamprecht & Seymour, 2010).

However, several points are debatable and should be addressed. It appears unlikely that the droplets described by Lamprecht and Seymour (2010) were actually water. First, the analysed plant was placed under a respiratory hood which can be expected to limit the evaporation. Second, droplet formation on the appendix has been observed in other species too and has been identified as odoriferous secretions (Kakishima et al., 2011; Shirasu et al., 2010; personal observation CC).

Moreover, heat loss is generally not detected in the appendix before anthesis. The cuticle effectively insulates the appendix and prevents evaporative heat loss. Therefore, heat loss through convection/radiation begins only once anthesis and heat production starts. But even then, convection/radiation is probably not the main cause of heat loss. Instead, some of the emitted scent compounds, for example trimethylamine and oligo dimethylsulphides, have a very high vapour pressure. It must therefore be considered that scent volatilisation itself is either the main or at least an important contributor to the evaporative cooling, as the appendix is designed to volatise significant amounts of scent compounds in a short time. It is likely not just a coincidence that species that emit large proportions of the highly volatile trimethylamine are the species that cool below ambient temperature during anthesis, such as *A. brachyphyllus* (appendix) and *A. gigas* (Kakishima et al., 2011). This is more likely to contribute to a temperature decrease than passive evaporation, convection or radiation.

It should also be considered that most *Amorphophallus* species have a short pistillate phase, often less than 12 h. Ample amounts of scent compounds are discharged within a short time and it needs to be investigated if this requires an active, energy-consuming release system, as well as the formation of secretory channels shortly before and during anthesis. The morphological changes preceding and during scent release have been investigated in *Sauromatum venosum*, another thermogenic Aroideae and important morphological changes accompanying anthesis were described by Skubatz et al. (1993, 1995) and Skubatz and Kunkel (1999). Moreover, Terry et al. (2016) studied the relationship between temperature elevation, increased respiration rates and the formation and emission of volatiles in *Macrozamia* Miq. cycad cones. These authors concluded that the energetically expensive synthesis and release of monoterpenes – and not thermogenesis – is at the origin of the respiratory metabolic burst (Terry et al., 2016).

It is therefore reasonable to consider that respiration rates during anthesis could be elevated through metabolic activities, such as the biosynthesis of some scent compounds, the formation of secretory channels, and the release of scent compounds. Consequently, if the temperature elevation is low and the respiration rates are high, it does not forcefully imply a strong heat loss (Lamprecht & Seymour, 2010; Seymour, 2010). Instead, it might signify that other metabolic activities linked to anthesis lead to the elevated respiration rates.

Moreover, the temperature measurements are taken at 2–3 mm depth, and it is unlikely that the tissue loses heat so quickly. Therefore, even if temperature measurements are not fully accurate, they should still represent a valid approximation.

Last but not least, there are potential pitfalls in the comparison of temperature measurements from a multitude of morphologically different species. This is usually 'overcome' by the comparison of mass-specific respiration rates (Seymour, 2010). However, in our case, many flowering events were unique, making it impossible to take the according data without damaging the inflorescence. That said, the case of *A. schmidtiae* demonstrates that the impact of morphology appears to have limits of its own. Plants grown from clonally propagated tubers of *A. schmidtiae* yielded two distinctly different temperature patterns, despite their identical floral morphology and genetics. This phenomenon requires additional research. Nonetheless, it clearly demonstrates that morphology alone cannot account for the varied temperature patterns.

These morphological aspects deserve closer observation and have to be addressed in forthcoming studies. The interplay between metabolism, scent production and release, temperature elevation, thermo-regulation and morphology appears to be more complex than previously assumed. In the meantime, temperature measurements have provided a reliable approximation of thermogenesis in several studies (Seymour et al., 2004; Seymour, Gibernau, & Itoh, 2003) and have been widely used in multiple systems (Hoe et al., 2020; Marotz–Clausen et al., 2018; Prieto & Cascante–Marín, 2017; Sayers et al., 2020; Skubatz et al., 2019). Temperature measurements also present some experimental advantages, such as the possibility of simultaneous individual recording of different floral organs or tissues, and the ease of use, particularly when dealing with large inflorescences. From an ecological perspective, detecting temperature elevation is particularly informative when considering biotic interactions among species, such as heat reward to pollinators. Based on these theoretical and practical considerations, this study does not quantify all the physiological variables involved in thermogenesis and instead uses detailed temperature measurements to approximate thermogenic activity in the plants surveyed. The evolution of thermogenesis in plants remains a hot topic.

CONCLUSIONS

The variation in thermogenic patterns and temperature fluctuation in *Amorphophallus* easily outranks the variation

in any other thermogenic plant group studied so far. Our results indicate that thermogenesis evolved only once early in the evolutionary history of *Amorphophallus* and, despite the loss of this function in several lineages, closely related species tend to display a similar temperature elevation. We also show that thermogenesis is at least partly decoupled from evolutionary success, as it did not influence the rate of species diversification within the genus. Although neither phylogenetic relationships nor thermogenic activity are the primary correlates of floral morphology in *Amorphophallus*, we find that thermogenic capacity is associated to some degree to inflorescence types that are likely adapted to beetle pollination. Yet, the phenomenon is only partly understood and the exact functional role that thermogenesis may have in pollinator attraction remains to be further clarified. Additional measurements and observations are required, particularly concerning the identity and the behaviour of visiting and pollinating insects.

Amorphophallus provides an exciting window into the evolution and natural history of thermogenesis in plants. However, as long as accurate data about species distribution, ecological niche and their pollinators are lacking for most *Amorphophallus* species, the evolution of thermogenesis will remain only partly understood. In addition to increased sampling and temperature measurements under controlled conditions, extensive field observations are also crucially needed to fill the remaining knowledge gaps. Unfortunately, gaining such understanding from natural ecosystems represents a race against time. Indeed, 12 out of the only 16 species assessed by the International Union for Conservation of Nature are threatened or nearly threatened, of which four species are classified under the highest threat category 'Critically Endangered', and two species have too sparse data for being reliably categorised (IUCN v. 2022–2; https://www.iucnredlist.org; accessed in April 2023). Further assessments of the remaining species are urgently needed to guide effective conservation strategies and safeguard the future of these unique and fascinating plants.

MATERIALS AND METHODS

Quantitative measurements of thermogenic activity

We recorded the temperature elevation above room temperature, a proxy for thermogenic activity, of 119 individuals representing 80 *Amorphophallus* species (Data S1) using an Extech SD200 3-channel- or an Extech SDL200 4-channel data logging thermometer (accuracy ±1°C). Fine-wired type K thermocouples of less than 1 mm diameter width were chosen in order to keep the plants as intact as possible. Most measurements took place between 2014 and 2020 in a climatised room in the Institute for Plant Science and Microbiology, Hamburg, Germany, after pilot trials also carried out at the Gothenburg Botanical Garden, Gothenburg, Sweden. Thirty of the measurements were performed in Cairns, Australia, under similar conditions. Most plants were either placed in a shaded and climatised room with largely constant ambient temperature, usually in the range of 20–25°C, or in a shaded office under low-temperature fluctuations. Six plants (*A. gallaensis*, *A. josefbogneri*, *A. ochroleucus*, *A. palawanensis*, *A. pilosus* and *A. thaiensis*) were directly analysed in the greenhouses where it was ensured that no direct sunlight reached the plants. Lastly, two plants (*A. polyanthus* and *A. terrestris*) were analysed within a terrarium under similar conditions as in the greenhouse (~85% humidity) around 20°C. Many investigated plants originate from cultivated material derived from the former research collection from Wilbert Hetterscheid in Leiden, or from the collection from Steve Jackson, a retired horticulturist from Cairns Botanic Gardens, Australia. Although cultivated in botanical gardens, they represent original *in situ* collections. Plants for investigation were chosen opportunistically, depending on the formation of an inflorescence. It must be noted that many flowering events were unique opportunities as several of our studied species rarely flower. This unpredictability in flowering, combined with the complexity and costs of the equipment used, jointly explains why our measurements took 7 years.

The thermocouples were inserted at ~2–3 mm depth in the middle of the pistillate (female) zone, the staminate (male) zone and the lower third of the appendix, usually at their broadest zone. The fourth thermocouple recorded room temperature as a reference. Measurements were taken every 5 min, starting at the onset of anthesis. Additionally, in eight species (Data S2 embedded movie), thermal images were shot using an InfraTec mobileIR E9 thermal camera (accuracy ±2°C). Emissivity was set to 0.98 and one image was taken every 5 min. The spathe of the inflorescence was removed either partially or totally, for visualisation purposes. Thermal imaging served to identify the thermogenic zones and to detect putative spatial dynamics not detectable by the thermocouples. Beyond that, thermal imaging was not used for analytical purposes. In eight selected species *A. albispathus*, *A. lewallei*, *A. paeoniifolius*, *A. prainii*, *A. schmidtiae* pattern 1, *A. schmidtiae* pattern 2, *A. tuberculatus*, *A. yunnanensis*, representing the four subgenera, thermal images were assembled to generate time-lapse movies (Data S2 embedded movie).

Several species were analysed in multiple replicates, either of the same clonally propagated plant or of different individuals of a species, in order to assess both the reproducibility and the variability of the thermogenic patterns within species (Data S1).

Time-series clustering

Thermogenesis is a dynamic phenomenon, and we recorded it as time series, that is temporal measurements of a continuous value (temperature) in the floral organs. Therefore, we used time-series clustering to classify species according to their thermogenic pattern, based on the full length of the temperature series rather than on punctuated events such as maximum temperature or temperature range. We applied shape-based clustering, an approach that aligns the shapes of two time series by warping some of their points along the time axis in order to find the optimal path between them (Aghabozorgi et al., 2015). We performed three different hierarchical clustering analyses, based on the time series of (1) the male zone, (2) the appendix, and (3) the male zone and appendix combined (i.e. multivariate time series). Clustering was performed using the Dynamic Time Warping (DTW) distance implemented in the R package dtwcluster (Sardá-Espinosa, 2019). The analyses were performed on: (i) the complete dataset with 119 time series, including multiple measurements available for 20 species; in some cases, based on clonally propagated plants, in

some cases different specimens of a species; and (ii) a dataset with 80 time series, containing a single individual per species, usually the individual reaching the highest temperature unless its time-series was incomplete due to measurements starting after the onset of thermogenesis or ending too early. Prior to the analysis, all variables were smoothed using the *loess* function in the R package stats.

In order to be used in downstream analyses, the output clusters from the clustering analysis were subsequently converted to dendrograms using the function *as.dendrogram* from the R package stats (version 3.6.2), and exported to the newick format, using the function *as.phylo.dendrogram*, from the R packages ape. Additionally, because the complete temperature time series of the appendix and male zone cannot be used in phylogenetic comparative analyses, we summarised them by computing for each of the 80 species the maximum temperature (later in the text referred to as peak temperature) of the appendix and male zone.

Phylogenetic analyses

We inferred a new time-calibrated molecular phylogeny of *Amorphophallus* including 157 species, that is 67% of the described taxonomic diversity of the genus (Claudel et al., 2017) and three outgroup species from other genera of the Araceae family, *Anchomanes difformis* (Bl.) Engl., *Gonatopus angustus* N.E. Br. and *Hapaline* sp. We used the molecular data from Claudel et al. (2017), which included one nuclear (*ITS1*) and two chloroplast (*rbcL* and *matK*) genes. In contrast to Claudel et al. (2017), who concatenated the three DNA markers and did not estimate divergence times, here we used the partitioned dataset to jointly estimate the tree topology and divergence times in a Bayesian framework using BEAST v2.5.0 (Bouckaert et al., 2014). The alignment was partitioned into nuclear and chloroplast genes, with a GTR + G substitution model for each partition. We applied a birth–death tree prior and an uncorrelated log-normal clock.

In order to produce a time-calibrated phylogeny, we implemented three secondary calibration points using the age estimates of Nauheimer et al. (2012) on the following nodes: (1) crown *Amorphophallus*: 95% HPD (highest posterior density) = 9.76–40.43; (2) *Amorphophallus* + *Hapaline*: 95% HPD = 47.42–68.23 and (3) the root node (node 38 of Nauheimer et al., 2012): 95% HPD = 77.1–97.03. All calibrations were set with a uniform prior to the 95% HPD interval. Convergence of the Markov chains Monte Carlo (MCMC) was assessed in Tracer v1.7.1 (Rambaut et al., 2018). We removed the first 10% of the MCMC samples as a burn-in and produced the maximum clade credibility tree using BEAST plug-in logAnalyser. The phylogeny and traits were visualised using the R package ggtree (Yu et al., 2017).

Diversification rate analyses

We investigated the species diversification dynamic of *Amorphophallus* by testing for constant diversification versus time-varying models. We used RPANDA (Morlon et al., 2016) to fit ten birth–death models, including a pure birth model, constant rate birth–death (BD) and other BD models with λ and/or μ varying exponentially or linearly as a function of time. We selected the best-fit model using AIC. However, because the temporal dynamics of diversification rates has been shown to suffer from unidentifiability issues (Louca & Pennell, 2020), we also estimated the 'pulled speciation rate' on a time grid using the R package castor (Louca & Doebeli, 2018). The pulled speciation rate is fully identifiable and corresponds to the speciation rate under zero extinction and complete sampling, meaning that in the presence of extinction or missing taxa, its value is lower than the speciation rate (Helmstetter et al., 2022) and can be informative or overall rate variation (Louca & Pennell, 2020).

Additionally, we tested whether thermogenesis impacted diversification rates in *Amorphophallus*. We compared character-dependent and character-independent models of diversification using the R package HiSSE (Beaulieu & O'Meara, 2016). We fitted a Hidden State Speciation and Extinction model where speciation and extinction rates are allowed to differ between thermogenic and non-thermogenic species, or due to another, hidden trait. This has been shown to reduce the risk of finding spurious evidence of trait-dependent diversification (Beaulieu & O'Meara, 2016). We compared the HiSSE model against a character-independent model and performed model selection using the AIC criterion.

Evolution of thermogenesis

To explore the evolutionary history of thermogenesis in *Amorphophallus* we performed ancestral state estimation for the 80 species with available temperature data. We carried out two sets of analyses with thermogenesis considered first as a continuous trait (peak temperature during anthesis in °C) and secondly as a binary trait (present/absent). For the latter, given the uncertainty of 1°C in the thermometer, species were coded as thermogenic only if they displayed >1.5°C heating above room temperature during anthesis in at least one of the two heat-producing parts of the inflorescence. All analyses were performed in the R package phytools (Revell, 2012). First, stochastic character mapping was done using the *make.simmap* function with 1000 simulations. Posterior probabilities of each state (thermogenic or not thermogenic) were plotted on the phylogenetic tree using the *densityMap* function. For the two continuous variables (peak temperature in the male part and in the appendix), we used maximum likelihood implemented in the function *contMap* to infer ancestral states at nodes and paint the inferred trait history along the phylogenetic tree.

In addition, we estimated the degree of phylogenetic conservatism of thermogenesis by computing Pagel's lambda (Pagel, 1999) for peak temperature of the male zone and of the appendix using the *phylosig* function in phytools. Departure from the null hypothesis of no phylogenetic signal ($\lambda = 0$) was tested using a Likelihood Ratio Test.

Determinants of thermogenesis

Thermogenesis has been associated with insect pollination (e.g. Seymour & Matthews, 2006). More particularly, beetle pollination has been reported to be characteristic for intensely thermogenic flowers with floral chambers (Bernhardt, 2000). Though most *Amorphophallus* species attract a wide array of arthropods (Claudel, 2021), beetles appear to be their main pollinator group (Moretto et al., 2019). Therefore, to test whether thermogenesis is associated with floral morphological traits, we scored for all species included in the phylogeny a matrix of 10 quantitative variables describing the main elements of the inflorescence (height and radius of the appendix and of the male zone, peduncle length and diameter, spadix length, spathe length) as well as pollen size. Some of these variables, for instance, appendix and male zone parameters are directly related to thermogenesis whereas others are part of the pollination system. For example, beetle pollination is associated with floral chambers and inflorescence robustness (Bernhardt, 2000; Johnson & Schiestl, 2016; Kevan & Baker, 1983), represented here by spathe parameters and peduncle diameter. Moreover, the length of the peduncle might be related to the flight ability of a pollinating insect. Lastly, pollen size is generally associated with biotic and abiotic parameters, such as wind- and insect

pollination as well as the feeding behaviour of the pollinating insects (Ackerman, 2000; Hao et al., 2020). Considering the significant size spread of pollen grains within *Amorphophallus*, ranging from 25 to 90 μm (Punekar & Kumaran, 2010; Ulrich et al., 2017; van der Ham et al., 1998), pollen size might be a significant variable.

To assess whether the evolution of inflorescence size and shape is more tightly linked to thermogenesis or to shared ancestry (Q3), we estimated Pagel's lambda for each of the 10 morphological traits, first on the phylogeny and second on the cluster dendrogram which reflects the degree of closeness in the thermogenic pattern. Tests for the null hypothesis of no phylogenetic signal were performed using a likelihood ratio test. For the analysis on the phylogeny, we took into account phylogenetic uncertainty by computing Pagel's lambda on a set of 1000 phylogenetic trees randomly sampled from the posterior distribution of trees. The larger number of species included in the phylogenetic tree compared to the cluster dendrogram (157 versus 80) hinders a direct comparison of Pagel's lambda values. To address this, we repeated the analysis with the phylogenetic tree pruned to keep only the species included in the cluster dendrogram.

We then built a multivariate regression model to evaluate whether thermogenic activity can be predicted from morphological traits. We used phylogenetic mixed models to model thermogenic activity as a function of morphological traits, using the Bayesian implementation in the R package mcmcglmm (Hadfield, 2010). To account for phylogenetic non-independence, phylogenetic relationships were included as a random variable. Two variables with a high proportion of missing data (pollen size and spadix width) were discarded from the analysis so that in total, the regression included 8 variables and 67 species. We carried out two analyses where the response variable was peak temperature in the appendix and in the male zone, respectively. The glmm analyses were run for 20 million MCMC generations, sampling every 12 000 generations. After discarding a burn-in of 120 000 generations, we checked the convergence of the MCMC chains. It would have been desirable to run a mixed model that also included geography as an explanatory variable, in order to test whether the intensity of thermogenic activity differs significantly between Asian, African and Malagasy species. However, there was insufficient statistical power in our dataset to test this hypothesis, given that only three African species and two species from Madagascar could be included in the phylogeny together with 43 Asian species.

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AUTHOR CONTRIBUTIONS

CC and AA initiated the project and carried out pilot measurements. CC collected the data. OL and DS analysed the data. CC, OL, DL, SLY and AA wrote the manuscript.

CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are deposited as temperature curves in the supporting information; original measurements are available from the corresponding author upon request.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Data S1. Time series of appendix and male zone temperatures for the 119 measured individuals representing 80 *Amorphophallus* species. Each time series is represented by two graphs: the first graph displays the temperature measurement and the second graph shows the absolute temperature increase.

Data S2. Time-lapse movies from *A. albispathus*, *A. lewallei*, *A. paeoniifolius*, *A. prainii*, *A. schmidtiae* pattern 1, *A. schmidtiae* pattern 2, *A. tuberculatus*, *A. yunnanensis* are accessible in the embedded movie file.

Data S3. Hierarchical dendrograms obtained from the clustering of the time series of appendix and male zone separately or combined, with 119 individuals (including replicates) or 80 (one individual per species).

Figure S1. The two cluster dendrograms based on the univariate time clustering analyses from the male zone and the appendix are broadly similar. Both display two main clusters, one containing species with medium to high-temperature increase and a second cluster consisting of weakly or non-thermogenic species.

Figure S2. Time calibrated molecular phylogeny of *Amorphophallus* inferred in BEAST. Node labels indicate posterior probabilities. Time in million years is shown in the horizontal axis.

Table S1. Comparison between the Hidden State Speciation and Extinction (HiSSE) and character-independent (CDI2) models of diversification.

Table S2. Values of Pagel's lambda for morphological variables estimated both on the cluster dendrogram of thermogenic activity and on the phylogenetic tree (with all species or pruned to the same tips as the dendrogram).

Table S3. Regression coefficients from the generalised-linear-mixed-model regression between the thermogenic activity of the male zone and appendix parts and morphological variables.

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