IDENTIFIABILITY OF PHYLOGENETIC LEVEL-2 NETWORKS UNDER THE JUKES-CANTOR MODEL

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Abstract. We investigate which evolutionary histories can potentially be reconstructed from sufficiently long DNA sequences, by studying the identifiability of phylogenetic networks from DNA sequence data generated under models of molecular evolution. While the identifiability of phylogenetic trees and level-1 networks, those with non-overlapping reticulation cycles, has been previously established, less is known about more complex network topologies. In this work, we extend identifiability results to broader classes of networks that include pairs of tangled reticulations.

Our main result establishes that binary semi-directed level-2 phylogenetic networks are generically identifiable under the Jukes–Cantor model, provided they are triangle-free and strongly tree-child. We also strengthen existing identifiability results for level-1 networks, showing that the number of reticulation nodes is generically identifiable under the Jukes-Cantor model.

In addition, we present more general identifiability results that do not restrict the network level at all and hold for the Jukes-Cantor as well as for the Kimura-2-Parameter model. Specifically, we demonstrate that any two binary semi-directed networks that display different sets of 4-leaf subtrees (quartets) are distinguishable. This has direct implications for the identifiability of a network's reticulated components (blobs). We show that the tree-of-blobs of a network, the global branching structure of the network, is identifiable, as well as the circular ordering of the subnetworks around each blob, for networks in which edges do not cross and taxa are on the outside.

Keywords. Phylogenetic network; identifiability; reticulate evolution; tangled reticulations; level-2; Markov model; phylogenetic invariant.

1. Introduction

Reticulate events such as hybridization, horizontal gene transfer, and recombination are crucial in understanding the evolutionary histories of many classes of organisms (Arnold, 1997; Barton, 2001; Mallet, 2005, 2007; Keeling and Palmer, 2008; Soucy et al., 2015), including bacteria (e.g. Diop et al., 2022), viruses (e.g. Worobey et al., 2008; Pekar et al., 2021; Jiao et al., 2024), plants (e.g. Rieseberg, 1997; Wang et al., 2024), fish (e.g. Meier et al., 2019), birds (e.g. Taylor and Larson, 2019), and primates (e.g. Patterson et al., 2006; Green et al., 2010; Raghavan et al., 2014). Indeed, due to the importance of such events, phylogenetic networks have emerged as a fundamental framework for modeling evolutionary relationships that involve reticulate events where lineages merge (Nakhleh, 2010; Bapteste et al., 2013; Kong et al., 2022, 2025).

In short, phylogenetic networks are graphs used to represent evolutionary relationships between different taxa, see Figure 1 for an example. A *graph* consists of nodes connected by edges.

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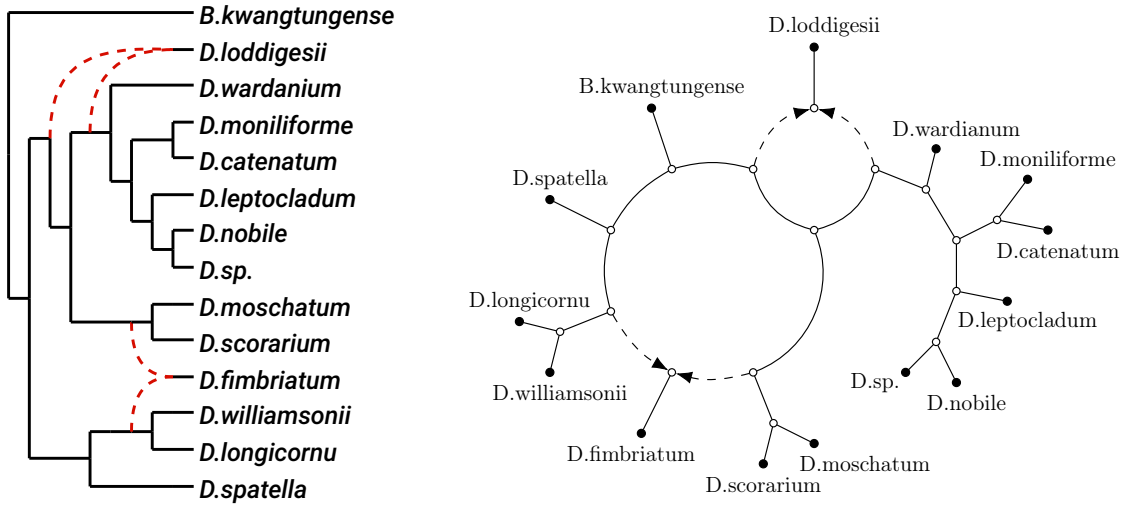

FIGURE 1. Left: A directed level-2 phylogenetic network of orchids, from Wang et al. (2024), where the directions go from left to right. Right: Its semi-directed network, obtained by suppressing the root and only letting the dashed reticulation edges keep their direction.

The nodes may be observed in the data (the leaves, representing taxa) or inferred by the analysis (internal nodes, representing ancestors). The edges may be directed (from ancestor to descendant) or undirected. Nodes may have more than one parent, making the relationships reticulate. Such nodes are called *reticulation nodes*.

This work assumes that the observed data are a set of aligned DNA sequences. These sequences changed (evolved) from a common ancestor, providing evidence that may allow the history to be reconstructed. The objective of our analysis is to determine whether such sequences contain enough evidence to infer a graph representing an evolutionary history or phylogeny when reticulate events are present.

Indeed, as network structures increase in complexity, it may become impossible to determine the network that produced a given data set, no matter the length of the sequences. Therefore, a major question of study in this area is: what network structures are identifiable, i.e., can potentially be uniquely recovered from sufficiently long DNA sequences, assuming a certain model of DNA evolution? This question is important for the development of consistent phylogenetic network reconstruction methods, i.e., methods that are guaranteed to reconstruct the correct network when given perfect data, such as the recently developed NANUQ (Allman et al., 2019), TINNIK (Allman et al., 2024a), NANUQ$^+$ (Allman et al., 2025) and SQUIRREL (Holtgrefe et al., 2025a) methods.

In this paper, we consider identifiability of phylogenetic networks from site-pattern probabilities under a model assuming a Jukes-Cantor (JC) or Kimura-2-Parameter (K2P) substitution process on each network edge, with no incomplete lineage sorting, where, at reticulation nodes, each site is independently inherited from one of the parent nodes under inheritance probabilities. Although this is a heavily simplified model, we see it as a major first step towards studying identifiability under more complex models.

So far almost all identifiability results and consistent reconstruction methods for phylogenetic networks have been restricted to the class of level-1 networks. The major breakthrough presented in this paper is to show identifiability results for level-2 networks, which are significantly more complex than level-1 since they allow pairs of "tangled" reticulate events, see Figure 2. This can

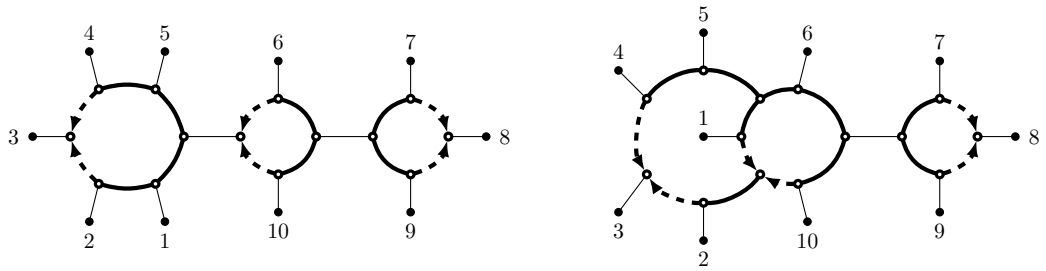

FIGURE 2. Illustration of the difference between level-1 (left) and level-2 (right) semi-directed phylogenetic networks. Nontrivial blobs are indicated in bold. Dashed arcs are reticulation edges and the nodes they point at are reticulation nodes.

be made more precise by using the term *blob*, a maximal subnetwork that cannot be disconnected by deleting a single edge. A blob is *trivial* if it consists of a single node. In a level-1 network, each nontrivial blob contains exactly one reticulation node and hence consists of a single cycle. Hence, any two cycles in a level-1 network must not overlap. Unfortunately, this restriction excludes many realistic evolutionary scenarios (e.g. Ge et al., 1999; Westenberger et al., 2005; Sessa et al., 2012; Raghavan et al., 2014; Szöllősi et al., 2015; Wang et al., 2024). For example, consider the phylogenetic networks for orchids depicted in Figure 1. The network to the left is directed. Since the root location is not identifiable under many models, we will consider *semi-directed* networks (see the network to the right in Figure 1 and Solís-Lemus and Ané (2016)). Such networks can often be rooted in practice using an outgroup. The networks in Figure 1 are not level-1, since they contain overlapping cycles. They, in fact, belong to a larger class of networks called *level-2* networks, which are binary networks where each blob contains at most two reticulation nodes. Level-2 networks can be seen as networks with isolated reticulations and pairs of tangled reticulations. Tangled reticulations lead to major mathematical and computational challenges especially when considering identifiability.

Roughly speaking, our main result is that binary semi-directed level-2 phylogenetic networks are identifiable from site-pattern probabilities under a Jukes-Cantor substitution process with inheritance probabilities, assuming generic parameters and under some technical conditions on the network topology, see Theorem 3.2 for a precise statement.

The main breakthrough in Theorem 3.2 is that it allows for tangled pairs of reticulate events. In addition, it does not require the number of reticulate events to be known. On the other hand, more complicated structures than level-2 are not allowed, such as triples of reticulations that are all tangled in a single blob. There are two additional technical restrictions on the networks. The first is the triangle-free assumption, which forbids reticulate events between a node and a direct ancestor of that node (leading to a length-3 cycle or *triangle*). Previous results either exclude these scenarios or in some cases show that current methods are unable to identify them (Gross and Long, 2018; Baños, 2019; Allman et al., 2022; Xu and Ané, 2023). The second is the strongly tree-child condition that forbids a reticulate event to be directly descendant another reticulate event (which is known to be unidentifiable (Sullivant, 2025)) and nodes that are the parent of two reticulate events (less likely scenarios for certain types of data (Kong et al., 2022)).

Along the way, as part of our proofs, we prove several other other results that are interesting in their own right and hold for networks of any level. First of all, we show that under JC and K2P, if two networks have different sets of displayed quartet trees, then they are distinguishable. This has consequences for the identifiability of the *tree-of-blobs*, the tree obtained from a network

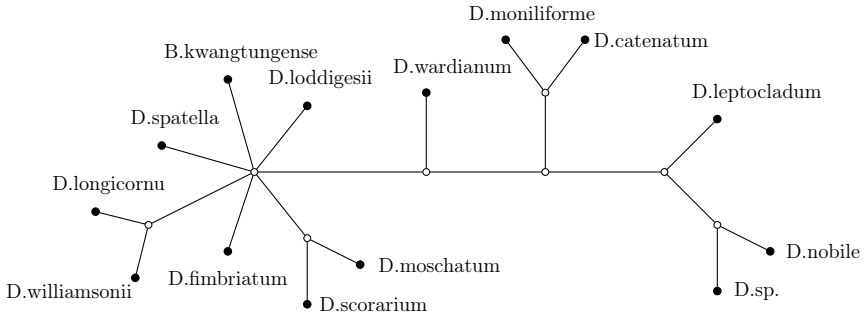

FIGURE 3. The tree-of-blobs of the semi-directed level-2 network on 14 orchids from Figure 1.

by contracting each blob to a node (Gusfield et al., 2007; Allman et al., 2023, 2024a), capturing the global branching structure of a phylogenetic network, see Figure 3. It is important to note that reconstructing a tree-of-blobs is fundamentally different from reconstructing a phylogenetic tree. However, by combining our result with results from Allman et al. (2023); Huber et al. (2025); Rhodes et al. (2025), it follows that under JC and K2P the tree-of-blobs of any binary semi-directed network is identifiable as well as the circular ordering of the subnetworks around certain blobs (see Theorem 3.3).

We also improve the results of Gross et al. (2021), by strengthening the identifiability results for binary level-1 networks under JC. We show that we do not need to assume that the networks are triangle-free, as long as we do not need to identify the reticulation node in a triangle. Moreover, we show that one can even distinguish between level-1 networks with different numbers of reticulate events (see Theorem 3.1). This could be important since the number of reticulate events is in practice usually not known in advance. In particular, it means that we can distinguish between tree-like and level-1 network-like evolution. We show that this even holds for level-2 and conjecture that it can be generalized to all levels.

1.1. **Related work on the identifiability of phylogenetic networks.** A prominent line of research focuses on network inference from gene tree distributions, where gene trees arise under the multispecies coalescent process (Yu et al., 2011). In this context, the problem of identifiability is analyzed using gene tree probabilities and related summary statistics such as quartet concordance factors. This line of research (Solís-Lemus and Ané, 2016; Baños, 2019; Allman et al., 2023; Rhodes et al., 2025; Allman et al., 2024b) eventually led to the identifiability of semi-directed level-1 phylogenetic networks up to the orientation of triangles, to the identifiability of the tree-of-blobs of general networks and of the circular ordering around certain blobs, from quartet concordance factors.

On the other hand, there have been a number of papers which specifically consider Markov models of evolution of aligned sequence data on a network. So far, such work on networks usually assumes that each column of the alignment evolves independently down a tree displayed by the network, contrasting with coalescent-based models which consider all trees.

Gross and Long (2018) proved, using algebraic geometry, that binary large-cycle networks, which are binary semi-directed phylogenetic networks with a single cycle of length greater than three, are generically identifiable under JC. Hollering and Sullivant (2021) used algebraic matroids to show that pairs of phylogenetic trees are generically identifiable for 2-tree CFN and Kimura 3-Parameter (K3P) mixtures, where each column evolves down one of two trees, and that binary large-cycle networks are generically identifiable under K2P and K3P. Gross et al.

(2021) showed that binary semi-directed level-1 networks are generically identifiable under JC, K2P and K3P, assuming the network is triangle-free and the number of reticulation nodes is known. While these works mostly focused on level-1 networks, Ardiyansyah (2021) explored an acutely restricted class of level-2 networks in a similar algebraic setting.

Furthermore, Francis and Moulton (2018) considered a different extension of the tree-based Markov model to networks, where blocks of columns of the alignment evolve down the same displayed tree and there is a fixed probability to start a new block evolving down another displayed tree. For this model they showed an identifiability result for the class of rooted binary tree-child networks with the same number of reticulation nodes, at the cost of fixing the parameter values of the evolutionary model.

Allman et al. (2022) use yet a different approach by showing that semi-directed level-1 networks are identifiable from sequence data via inter-taxa logDet distances under a model that includes incomplete lineage sorting and with some further restrictions on the networks.

1.2. **Outline of the paper.** In the next section, we cover preliminary background on networks, Markov processes on trees and networks, and identifiability including the algebraic approaches to identifiability we will use. In Section 2.2, we use linear invariants and inequalities to show that under JC and K2P, two networks that have different displayed quartet trees are distinguishable. In Section 2.3, we use phylogenetic invariants and matroids to distinguish certain pairs of quarnets (4-leaf subnetworks) which will be used in later proofs. In Section 2.4, we derive a new trinet inequality that can distinguish a 3-leaf tree from a level-1 or level-2 trinet (3-leaf subnetwork) under JC. Then, in the Results section, we put all these results together to prove our main results described above. We end with a Discussion section.

2. Materials & Methods

2.1. Preliminaries.

2.1.1. *Phylogenetic networks.* In this section, we introduce concepts from graph theory that we utilize to study phylogenetic networks. The *lowest stable ancestor* in a rooted directed acyclic graph is the lowest node through which all directed paths from the root to a leaf pass. The *in-degree* of a node v is the number of edges that is incident to v and directed towards v while the *out-degree* of v is the number of incident edges directed away from v.

Definition 2.1. A *directed (binary phylogenetic) network* N^+ on a set of taxa $\mathcal{X}$ is a directed acyclic graph where

(i) $\mathcal{X}$ is the set of nodes with out-degree zero and in-degree one (*leaves*);
(ii) there is a unique *root*, which has in-degree zero, out-degree two and is the lowest stable ancestor; and
(iii) all other nodes have in-degree one and out-degree two (*tree nodes*) or in-degree two and out-degree one (*reticulation nodes*).

The network N^+ is a *rooted (binary) phylogenetic tree* if the network does not contain any reticulation nodes. We call a non-leaf node in N^+ *internal* and an edge directed into a reticulation node a *reticulation edge*. A pair of reticulation nodes v, w is called *stacked*, if one is a child of the other.

Definition 2.2. A *semi-directed (binary phylogenetic) network* N on a set of taxa $\mathcal{X}$ is a partially directed graph that can be obtained from a directed binary phylogenetic network on $\mathcal{X}$ by removing edge directions of non-reticulation edges and suppressing the former root.

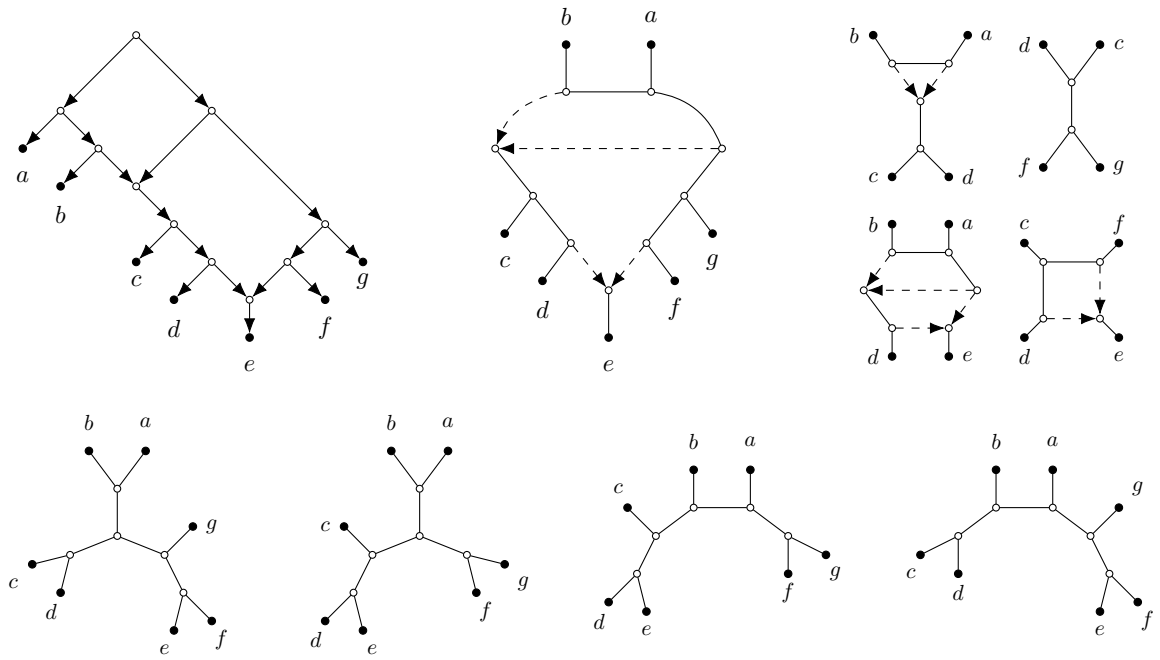

FIGURE 4. *Top left:* A directed phylogenetic network N^+ on $\mathcal{X} = \{a, b, c, d, e, f, g\}$. *Top middle:* The semi-directed phylogenetic network N obtained from N^+. *Top right:* Four of the quarnets induced by N. *Bottom:* The four unrooted phylogenetic trees on $\mathcal{X}$ displayed by N.

Reticulation nodes, reticulation edges, internal nodes, and leaves are defined analogously for semi-directed networks. A semi-directed network without reticulation nodes is called an *unrooted (binary) phylogenetic tree*. Such a tree is *displayed* by a semi-directed network on $\mathcal{X}$, if it can be obtained by deleting exactly one incoming reticulation edge per reticulation node, omitting the direction of the other incoming edge, and then exhaustively deleting out-degree-0 nodes not in $\mathcal{X}$ and suppressing degree-2 nodes. See Figure 4 for an example of all unrooted phylogenetic trees displayed by a semi-directed network.

A node v is *below* a reticulation node r in a semi-directed network N if there is a path from r to v in which all traversed reticulation edges are directed towards v. The node v is *directly below* r if v and r are also adjacent. Every *cut-edge* e of a (semi-)directed network, i.e. an edge whose removal disconnects a graph, induces a bi-partition $A \cup B$ of its leaves $\mathcal{X}$, in which case we say that e induces the *split* $A|B$. The split is *nontrivial* if both $|A|, |B| \geq 2$.

A *blob* of a (semi-)directed phylogenetic network is a maximal connected subgraph without any cut-edges. Such a blob is an m-blob for some positive integer m if the blob is incident to exactly m edges not in the blob. A blob is *trivial* if it consists of a single node. The unrooted (possibly non-binary) phylogenetic tree that is obtained from a semi-directed network by contracting every blob to a single node is called the *tree-of-blobs*.

Remark. Throughout this work we assume that semi-directed networks have no 2-blobs or parallel edges, since suppressing them does not affect the site-pattern probabilities under our considered models, see Sullivant (2025). Thus, all our identifiability results extend to the setting where such structures are allowed but one aims to identify the network with these structures suppressed.

Note that this property is specific to *equivariant models* (which include the JC and K2P models we consider) and does not extend to more general models such as the GTR model.

Definition 2.3. Let N^+ be a directed phylogenetic network (respectively, N a semi-directed phylogenetic network), and let k be some non-negative integer. Then, we call the network N^+ (respectively, N)

(i) *level-k* if there are at most k reticulation nodes in each blob of the network;
(ii) *strict level-k* if the network is level-k but not level-$(k-1)$;
(iii) *stack-free* if none of the reticulation nodes of the network are stacked;
(iv) *simple* if the network has a single non-leaf blob;
(v) *triangle-free* if the network contains no cycles of length three, where edge directions are disregarded;
(vi) *outer-labeled planar* if the network can be drawn in the plane without crossing edges and with the leaves on the outside.

Furthermore, N^+ is *tree-child* if every internal node has at least one child that is either a tree node or a leaf. N is *strongly tree-child* if every directed network from which N can be obtained (as in Definition 2.2) is tree-child. Holtgrefe et al. (2025b) showed that N is strongly tree-child if and only if each node with an outgoing edge also has two incident undirected edges.

To illustrate some of these definitions, consider Figure 4, which depicts a tree-child directed network N^+ and the strongly tree-child semi-directed network N that can be obtained from N^+. Note that, while the directed network N^+ is not the unique directed phylogenetic network from which N can be obtained, all possible rootings of N result in a tree-child network. Both networks are strict level-2, simple, triangle-free, stack-free and outer-labeled planar. By contrast, the semi-directed network in Figure 1 is not simple because every internal node not included in a cycle is also a blob, i.e., the network contains eight non-leaf blobs. Also note that our definition of a simple network is different from the definition of simple graphs in standard graph theory terminology.

Since outer-labeled planar, semi-directed networks can be drawn in the plane with leaves on the outside, traversing the boundary of such a drawing yields a *circular order* (i.e., an order up to reversal and cyclic permutations) of the leaves. In general, an outer-labeled planar network may have multiple such drawings, and consequently multiple such *congruent* circular orders. However, as shown by Rhodes et al. (2025), if the network is also simple, this congruent circular order is unique. For example, the network N in Figure 4 is congruent with only the circular order (a, b, c, d, e, f, g).

An *up-down path* between two leaves x_i and x_j of a semi-directed network is a path of k edges where the first l edges are directed towards x_i and the last $k-l$ edges are directed towards x_j, for some $0 \leq l \leq k$, considering undirected edges to be bidirected. Using this notion, we can formally define the *restriction* of a semi-directed network.

Definition 2.4. Given a semi-directed network N on $\mathcal{X}$ and $X \subset \mathcal{X}$ with $|X| \geq 2$, we define the *restriction of N to X* as the semi-directed network $N|_X$ obtained from N by taking the union of all up-down paths between leaves in X, followed by exhaustively suppressing all nodes of degree two, parallel edges and 2-blobs.

Intuitively, the restriction of a semi-directed network can be viewed as its induced subnetwork. If $|X| = 3$, $|X| = 4$ or $|X| = 5$, then we call $N|_X$ a *trinet*, *quarnet* or *quinnet* induced by N, respectively. See Figure 4 for an example of four quarnets induced by a semi-directed network. Proving identifiability by restricting to (level-2) trinets, quarnets, and quinnets will be a key idea in our proofs. Figure 5 shows all sixteen semi-directed simple level-2 quarnets, up to labeling

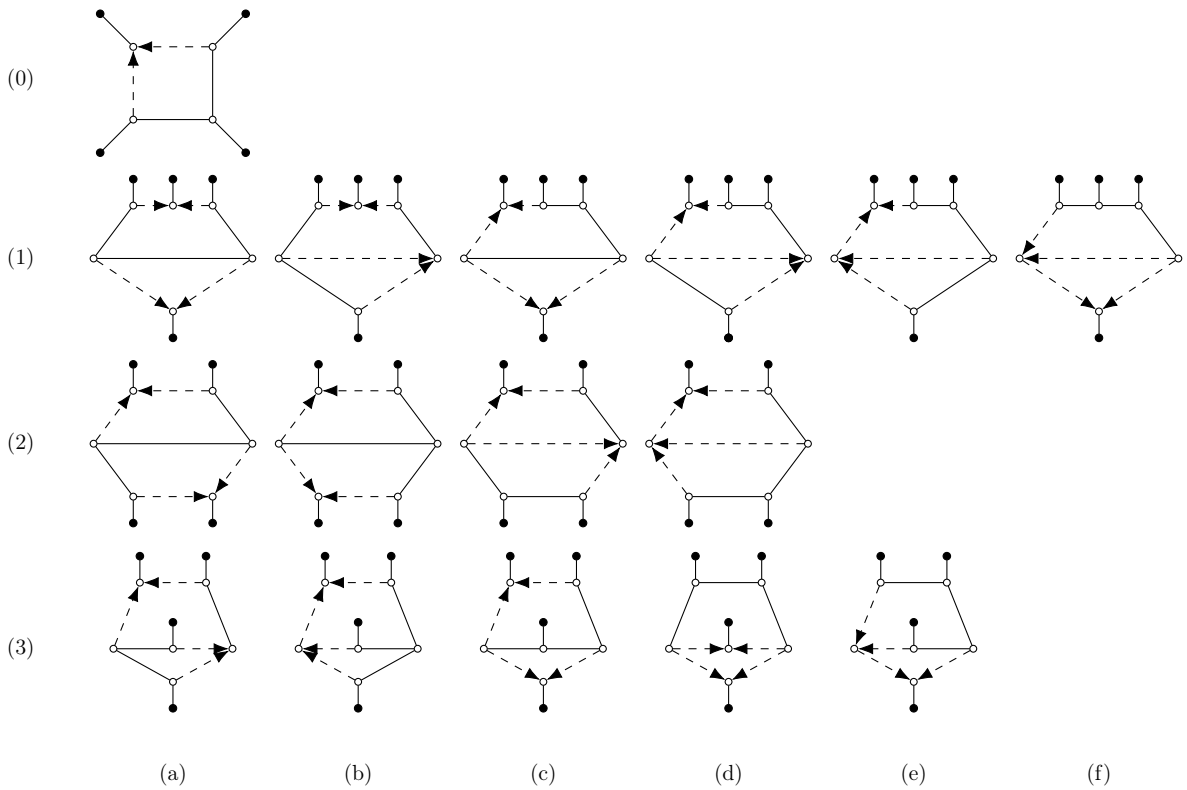

FIGURE 5. All sixteen semi-directed simple level-2 quarnets up to labeling the leaves.

the leaves. Whenever we refer to a quarnet of a specific type, we use the annotations as in this figure. For example, the quarnet of type 0 is the only simple quarnet that is strict level-1.

2.1.2. *Network-based Markov models.* In this paper, we consider network-based Markov models, which are described most naturally via a directed phylogenetic network. However, for reversible models, such as the Jukes-Cantor and Kimura 2-parameter models considered here, any two directed networks N_1^+ and N_2^+ that have the same semi-directed network N will yield the same set of probability distributions (see proof of Proposition 1 in Gross et al. (2021)). Thus, we can use directed networks and semi-directed networks interchangeably when describing model parametrizations.

Given a directed phylogenetic network $N = (V, E)$, we associate with each node $v \in V$ a random variable X_v with state space $\Sigma = \{A, G, C, T\}$, corresponding to the four DNA bases. The Jukes-Cantor (JC) and Kimura 2-parameter (K2P) substitution models then specify a distribution π at the root, and for each edge $e = (u, v)$, a transition matrix M^e governing the probabilities of nucleotide change from u to v (Jukes and Cantor, 1969; Kimura, 1980). This yields joint probability distributions $p_\omega(T)$ of a site pattern $\omega \in \Sigma^n$ observed at the leaves in $\mathcal{X}$ of each *switching* T of N. Here, a switching of N is a tree (possibly having degree-2 nodes and additional leaves not in $\mathcal{X}$) obtained by deleting exactly one incoming reticulation edge per reticulation node. See, for example, Gross et al. (2021) for more details.

Then, to derive the site pattern probability distribution of N, let k be the number of reticulation nodes of N and consider the set $\{T_1, \ldots, T_{2^k}\}$ of switchings of N. Assign a parameter $\delta_i \in (0, 1)$ to each reticulation node r_i with corresponding reticulation edges (e_i^0, e_i^1). Here, δ_i

(resp. $1-\delta_i$) is an *inheritance probability*, representing the probability that a particular site was inherited along edge e_i^0 (resp. e_i^1). For $j \in \{1,\dots,2^k\}$, let $\zeta_j \in \{0,1\}^k$ denote the indicator variable for the reticulation edges e_i^1 present in T_j. Then, the probability of observing site pattern ω in N is given by

$$p_\omega(N) = \sum_{j=1}^{2^k} \left(\prod_{i=1}^{k} \delta_i^{1-(\zeta_j)_i} (1-\delta_i)^{(\zeta_j)_i} \right) p_\omega(T_j).$$

Thus, for the stochastic parameter space Θ_N of transition matrices M^e, inheritance probabilities δ_i and root distribution π, we can view both the JC and K2P model on N as the image of the polynomial map

$$\begin{aligned} \psi_N : \Theta_N &\mapsto \Delta_{4^n-1} \\ (\pi, M^{e_1}, \dots, M^{e_m}, \delta_1, \dots, \delta_k) &\mapsto (p_\omega(N))_{\omega \in \Sigma^n}. \end{aligned}$$

The image of map ψ_N is called the *phylogenetic model associated to* N or a *phylogenetic model* if N is clear from the context, and is denoted $\mathcal{M}_N$.

For the Jukes–Cantor and Kimura 2-parameter models, a linear change of coordinates — the Fourier (or Hadamard) transform (Evans and Speed, 1993) — simplifies the parameterizations. We describe the parameterization of this model in Fourier coordinates $q_\omega(T)$ following Sturmfels and Sullivant (2005). First, we identify our state space Σ with the Klein four-group $\mathbb{Z}_2 \times \mathbb{Z}_2$ as follows: $A = (0,0)$, $G = (1,0)$, $C = (0,1)$, $T = (1,1)$. We associate four Fourier parameters with each edge $e \in E$, denoted by a_A^e, a_G^e, a_C^e and a_T^e. In addition, under the JC model we have that $a_C^e = a_G^e = a_T^e$, and under the K2P model we have that $a_G^e = a_T^e$. Furthermore, $a_A^e = 1$ for biological parameters. We assume throughout this paper that all other parameters are in the interval $(0,1)$, which corresponds to excluding zero and infinite branch lengths. Then, for site pattern $\omega = (g_1, g_2, \dots, g_n)$, the *Fourier coordinates* of $p_\omega(T)$ are given by

$$q_\omega(T) = \begin{cases} \prod\limits_{\substack{e \in E(T) \\ e \text{ induces the split } A|B}} a^e_{\sum_{i \in A} g_i} & \text{if } \sum\limits_{i=1}^{n} g_i = 0, \\ 0 & \text{otherwise} \end{cases}$$

where addition is in the group $\mathbb{Z}_2 \times \mathbb{Z}_2$. For any edge $e \in E$ which induces the split $A|B$, we also write Fourier parameter a_s^e as $a_s^{A|B}$ for all $s \in \Sigma$.

We conclude with the fact that for the JC and K2P models, all probability distributions in a phylogenetic model of a tree can be parameterized by a monomial with one parameter per edge in the tree. Therefore, distributions from a phylogenetic model of a network can be parameterized by a sum of monomials, one for each tree T_j, $j \in \{1,\dots,2^k\}$, weighted by the corresponding inheritance probabilities.

2.1.3. *Generic identifiability and distinguishability.* Our paper seeks to answer questions about the distinguishability and generic identifiability of phylogenetic networks, which we define and discuss here.

Definition 2.5. Let $\{\mathcal{M}_N\}_{N \in \mathcal{N}}$ be a class of phylogenetic network models and $\psi_N : \Theta_N \to \Delta_{4^n-1}$ be the parameterization map for a phylogenetic network model. Given two distinct networks N_1 and N_2 on the same set of n leaves, we say they are *distinguishable* if the set of numerical parameters in Θ_{N_1} that ψ_{N_1} maps into $\mathcal{M}_{N_2}$ and the set of numerical parameters in Θ_{N_2} that ψ_{N_2} maps into $\mathcal{M}_{N_1}$ both have Lebesgue measure zero. Then, the network parameter of a phylogenetic network model is *generically identifiable* if given any two distinct n-leaf networks $N_1, N_2 \in \mathcal{N}$, the networks N_1 and N_2 are distinguishable.

One of our main tools for proving identifiability results for a phylogenetic network model $\mathcal{M}_N$ will be to look at its *vanishing ideal* or *ideal of phylogenetic invariants*, defined as the set of polynomials

$$\mathcal{I}(\mathcal{M}_N) = \{f \in \mathbb{C}[p_\omega] : f(p_\omega) = 0 \ \ \forall p_\omega \in \mathcal{M}_N\}.$$

Here, we extend ψ_N to be a complex polynomial map ψ'_N by considering the polynomial ring $\mathbb{C}[p_\omega]$ over indeterminates p_ω, with $\omega \in \Sigma^n$. Clearly, each phylogenetic invariant f vanishes on all probability distributions of a given phylogenetic model. In particular, we have the following result that relates the existence of phylogenetic invariants to distinguishability (Sullivant, 2023).

Proposition 2.6. *Let $\mathcal{M}_{N_1}$ and $\mathcal{M}_{N_2}$ be two phylogenetic network models on the same number of leaves. Suppose that $\dim(\mathcal{M}_{N_1}) = \dim(\mathcal{M}_{N_2})$. If there exist a polynomial $f_1 \in \mathcal{I}(\mathcal{M}_{N_1}) \backslash \mathcal{I}(\mathcal{M}_{N_2})$ then networks N_1 and N_2 are distinguishable.*

It is useful to note that when $\mathcal{I}(\mathcal{M}_{N_1}) = \mathcal{I}(\mathcal{M}_{N_2})$, this is not enough to prove that models $\mathcal{M}_{N_1}$ and $\mathcal{M}_{N_2}$ are not distinguishable. In these cases, further methods, such as semi-algebraic constraints, are necessary to prove distinguishability or lack thereof.

Since computing phylogenetic invariants can be challenging, the following alternative method based on algebraic matroids — derived from Proposition 3.1 and Algorithm 3.3 in Hollering and Sullivant (2021) — can also be used to establish network distinguishability.

Proposition 2.7. *Let $\mathcal{M}_{N_1}$ and $\mathcal{M}_{N_2}$ be two phylogenetic network models on the same number of leaves, and suppose that $\dim(\mathcal{M}_{N_1}) = \dim(\mathcal{M}_{N_2})$. For $i \in \{1, 2\}$, let $J(\psi_{N_i})$ be the Jacobian matrix of ψ_{N_i} whose rows and columns are indexed by site patterns Σ^n and model parameters, respectively. Suppose there is a subset $S \subseteq \Sigma^n$ such that*

$$\operatorname{rank} J(\psi_{N_1})_S < \operatorname{rank} J(\psi_{N_2})_S$$

where $J(\psi_N)_S$ denotes the submatrix of $J(\psi_N)$ consisting of the rows indexed by S. Then, networks N_1 and N_2 are distinguishable.

Additionally, the following proposition shows that to prove distinguishability, it suffices to restrict to subsets of leaves. See Proposition 4.3 of Gross and Long (2018) for a related result.

Proposition 2.8. *Let N_1 and N_2 be two semi-directed networks on the same leaf set $\mathcal{X}$ under a certain phylogenetic model. Let $S \subset \mathcal{X}$, and suppose that $N_1|_S$ and $N_2|_S$ are distinguishable. Then, N_1 and N_2 are distinguishable.*

Proof. Restriction of a network model to a set of leaves S gives a linear map ϕ_S from the probability simplex $\Delta_{4^{|\mathcal{X}|}-1}$ to the probability simplex $\Delta_{4^{|S|}-1}$, that maps $\mathcal{M}_N$ onto $\mathcal{M}_{N|_S}$. This is because the restriction network is the network that is produced by summing out over the states of the leaves that are not in S. Since N_1 and N_2 are distinguishable, for $i, j \in \{1, 2\}$, $i \neq j$, the set of numerical parameters in Θ_{N_i} that ψ_{N_i} maps into $\mathcal{M}_{N_j}$ is a set A of Lebesgue measure zero. Then, $\phi_S(A)$ has Lebesgue measure zero because ϕ_S is linear. Thus, $N_1|_S$ and $N_2|_S$ are distinguishable too. □

With Proposition 2.8 in mind, our strategy for proving identifiability of the network parameter for our considered class of networks becomes clear. We will show distinguishability of many pairs of small networks, using different strategies including computing phylogenetic invariants, matroid calculations, and finding inequalities that can distinguish the small networks. Then we will show that arbitrary size networks can be distinguished by considering restrictions to small sets of leaves. In particular, in the next section we will provide semialgebraic sufficient conditions under which specific phylogenetic network models do not intersect which we will make use of repeatedly to prove distinguishability throughout the rest of the paper.

2.2. **Distinguishing Networks with Different Displayed Quartets.** This section builds on the linear invariants and inequalities developed by Allman et al. (2010, 2012) for tree mixture models to address identifiability of networks under the JC and K2P models. We show that networks of any level with different displayed quartets are distinguishable, and as a corollary, the tree-of-blobs of a network is also identifiable.

Proposition 2.9. *Consider the Fourier coordinates q associated to a quartet tree T with leaf set $\{1,2,3,4\}$ under the JC or K2P model. Then, the invariant*

$$q_{GGGG} - q_{GGTT} \tag{1}$$

evaluates to zero if T has split $12|34$ and is strictly positive if T has split $13|24$ or $14|23$.

Proof. In the first case, we have that

$$\begin{aligned} q_{GGGG} &= a_G^1 a_G^2 a_G^3 a_G^4 a_A^{12|34} \\ q_{GGTT} &= a_G^1 a_G^2 a_T^3 a_T^4 a_A^{12|34}. \end{aligned}$$

However, since $a_G^3 = a_T^3$ and $a_G^4 = a_T^4$ for JC and K2P we see that expression 1 evaluates to 0. On the other hand, for the tree with split $13|24$ we have

$$\begin{aligned} q_{GGGG} &= a_G^1 a_G^2 a_G^3 a_G^4 a_A^{13|24} \\ q_{GGTT} &= a_G^1 a_G^2 a_T^3 a_T^4 a_C^{13|24}. \end{aligned}$$

Then, since $a_G^3 = a_T^3$ and $a_G^4 = a_T^4$, and $a_A^{13|24} = 1$ for JC and K2P, expression 1 becomes $a_G^1 a_G^2 a_G^3 a_G^4 (1 - a_C^{13|24})$. Since $a_C^{13|24} \in (0,1)$, this is positive. A similar calculation shows that expression 1 is positive for distributions that come from the tree with split $14|23$. □

The proof of the following result is along similar lines to the proof of Proposition 2.9 and is thus deferred to the appendix.

Proposition 2.10. *Consider the Fourier coordinates q associated to a quartet tree T with leaf set $\{1,2,3,4\}$ under the JC or K2P model. Then, the invariant*

$$q_{GGGG} - q_{GGTT} - q_{GTTG} + q_{GTGT} \tag{2}$$

evaluates to zero if T has split $12|34$ or $14|23$ and is strictly positive if T has split $13|24$.

Theorem 2.11. *Let N_1 and N_2 be two binary semi-directed phylogenetic networks on the same leaf set $\mathcal{X}$. Let $\mathcal{M}_1$ and $\mathcal{M}_2$ be the corresponding sets of probability distributions produced by the Markov models on these networks under either the JC or K2P models. If there is a 4 element subset $S \subseteq \mathcal{X}$ such that the quarnets $N_1|_S$ and $N_2|_S$ have different sets of displayed quartets, then $\mathcal{M}_1 \cap \mathcal{M}_2 = \emptyset$.*

Proof. Due to Proposition 2.8 it suffices to assume that N_1 and N_2 are themselves quarnets, since if the marginalizations are disjoint then $\mathcal{M}_1 \cap \mathcal{M}_2 = \emptyset$. Let d_i be a distribution in $\mathcal{M}_i$ and note that it is a mixture of distributions from the switchings, or equivalently, from the displayed trees. Let D_i be the set of displayed quartets from network N_i. We will assume that $D_1 \neq D_2$, and show that $d_1 \neq d_2$, which proves $\mathcal{M}_1 \cap \mathcal{M}_2 = \emptyset$ if all mixing weights are positive.

Suppose that one of D_1 or D_2 is a singleton. After symmetry, we can assume that $D_1 = \{12|34\}$ and that D_2 contains one of the other quartet trees. By Proposition 2.9, expression 1 evaluates to 0 for d_1 and is positive for d_2, so $d_1 \neq d_2$.

Now assume that D_1 has two quartet trees, and D_2 has two or three trees. After symmetry, we can assume that $D_1 = \{12|34, 14|23\}$ and that $\{13|24\} \subseteq D_2$. Then, by Proposition 2.10, expression 2 evaluates to 0 for d_1, whereas it is positive for d_2. So, again, $d_1 \neq d_2$. □

In the following corollary we show that the tree-of-blobs of a semi-directed network of any level is identifiable under the JC and K2P models.

Corollary 2.12. *Let N_1 and N_2 be two binary semi-directed phylogenetic networks on the same leaf set X. Let $\mathcal{M}_1$ and $\mathcal{M}_2$ be the corresponding sets of probability distributions produced by the JC or K2P Markov models. If the trees-of-blobs of N_1 and N_2 are not isomorphic, then $\mathcal{M}_1 \cap \mathcal{M}_2 = \emptyset$.*

Proof. In Huber et al. (2025) it is shown that the tree-of-blobs of a semi-directed network of any level is uniquely determined by the nontrivial splits of its quarnets. Since N_1 and N_2 have non-isomorphic trees-of-blobs, for some 4 element set $S \subseteq \mathcal{X}$ the quarnets $N_1|_S$ and $N_2|_S$ do not induce the same nontrivial split (with possibly one of them not inducing a nontrivial split at all). Then, by Proposition A.1a, the two quarnets display a different quartet. The result then follows from Theorem 2.11. □

Corollary 2.13. *Let N_1 and N_2 be two binary semi-directed phylogenetic networks on the same leaf set $\mathcal{X}$. Let $\mathcal{M}_1$ and $\mathcal{M}_2$ be the corresponding sets of probability distributions produced by the JC or K2P Markov models. If N_1 and N_2 are both outer-labeled planar and they have different sets of congruent circular orders, then $\mathcal{M}_1 \cap \mathcal{M}_2 = \emptyset$.*

Proof. By the previous corollary, we may assume without loss of generality that N_1 and N_2 have the same tree-of-blobs. Then, for the two networks to have different sets of congruent circular orders, there must be a k-blob with $k \geq 4$ in the two networks which induces a different ordering of the subnetworks around it in N_1 than in N_2. Hence, we may assume without loss of generality that N_1 and N_2 are both simple networks, i.e. they consist of a single non-leaf blob. In Theorem 5.3 of Rhodes et al. (2025) it is shown that the circular order of a binary, simple, outer-labeled planar semi-directed phylogenetic network is uniquely determined by the circular orders of all its simple, outer-labeled planar quarnets. Since we assume that N_1 and N_2 are congruent with a different circular order, there is some 4-leaf set $A \subseteq \mathcal{X}$ such that $N_1|_A$ is congruent with a unique circular order and $N_2|_A$ is congruent with a different unique circular order or it is not congruent with a unique circular order. Then, by Proposition A.1, the two quarnets display different quartets. The result then follows from Theorem 2.11. □

2.3. **Distinguishing Small Networks using Invariants and Matroids.** In this section, we describe a lemma that distinguishes pairs of quarnets, which will later support identifiability of larger networks. The results in this section are computational: Lemma 2.14 is verified by a sequence of `Macaulay2` calculations (Grayson and Stillman, 1992). Direct calculations of the vanishing ideals of our considered models seem out of reach at present, so the computations instead rely on the underlying ideas of Propositions 2.6 and 2.7.

Lemma 2.14. *Let N_1 and N_2 be two binary level-2 semi-directed phylogenetic networks on the same leaf set $\mathcal{X}$. Let S be a 4 leaf subset of $\mathcal{X}$. Then, under the JC model:*

- *(a) if $N_1|_S$ and $N_2|_S$ are distinct quarnets of type 1b with a different leaf in the top middle in the planar representation of Figure 5, then N_1 and N_2 are distinguishable;*
- *(b) if $N_1|_S$ and $N_2|_S$ are distinct quarnets of type 2a in Figure 5, then N_1 and N_2 are distinguishable;*
- *(c) if $N_1|_S$ and $N_2|_S$ are distinct quarnets of type 3a, 3c or 3d in Figure 5, then N_1 and N_2 are distinguishable.*

Let $S = \{a, b, c, d, e\}$ be a 5 leaf subset of X. Then, under the JC model:

- *(d) if $N_1|_S$ and $N_2|_S$ are the two quinnets from Figure 6, then N_1 and N_2 are distinguishable.*

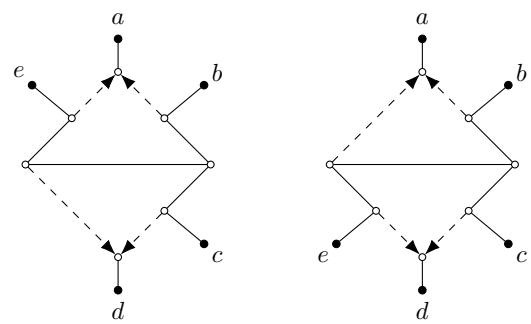

FIGURE 6. Two distinct simple, level-2 quinnets on leaf set $S = \{a, b, c, d, e\}$ that are distinguishable by Lemma 2.14d.

Proof. For each of these results, we may omit the $|_S$ notation, since it suffices to consider the case that S is the set of leaves of the networks under consideration, simply by marginalizing (see Proposition 2.8).

The computational proofs of these results were carried out in separate `Macaulay2` (Grayson and Stillman, 1992) files, with the parameterizations obtained using the algorithm available through (Grindstaff et al., 2025). Parts (a), (b) and (d) employ the matroid method of comparing the ranks of submatrices of the Jacobians from the two networks (see Proposition 2.7). For part (c) we instead compute low degree invariants of our networks using the *MultigradedImplicitization* toolbox (Cummings and Hollering, 2025) in `Macaulay2`, with distinguishability following from Proposition 2.6. In Appendix A, we discuss the used files and explain how each of these computations work in detail. □

2.4. **Distinguishing Three-Leaf Trees from Trinets.** In this section, we describe an inequality for the JC model that allows us to distinguish a three-leaf tree from a strict level-1 or level-2 trinet (as elsewhere in the paper, all parameters are assumed to belong to $(0, 1)$). This inequality helps us prove distinguishability results for many pairs of level-2 networks, since many level-2 networks have induced subnetworks that are trees.

For the Fourier coordinates q under the Jukes-Cantor model for a trinet, we write q_{110} as shorthand for any of the values $q_{CCA} = q_{GGA} = q_{TTA}$, since all of these values are the same under the Jukes-Cantor model. We define q_{101} and q_{011} similarly. We write q_{111} to denote any of $q_{CGT} = q_{CTG} = q_{GCT} = q_{GTC} = q_{TGC} = q_{TCG}$, as again all of these values are the same under the Jukes-Cantor model.

Proposition 2.15. *Consider the Fourier coordinates q associated to a binary, semi-directed level-2 trinet N under the JC model. Then the invariant*

$$q_{011}q_{101}q_{110} - q_{111}^2 \tag{3}$$

evaluates to zero if N is a three-leaf tree and is strictly positive if N is a strict level-1 or strict level-2 trinet.

Proof. Throughout the proof, we simplify the notation for the Fourier parameters as follows. In the description in Section 2.1.2, the parameters were notated as a_g^e, where e is an edge and g is a group element. However, in the Jukes-Cantor model, we have that $a_A^e = 1$, and $a_C^e = a_G^e = a_T^e \in (0, 1)$. Hence, we use a single letter to denote the parameter value $a_C^e = a_G^e = a_T^e$, and we use a different letter $a, b, c, \ldots$ for each edge in the network.

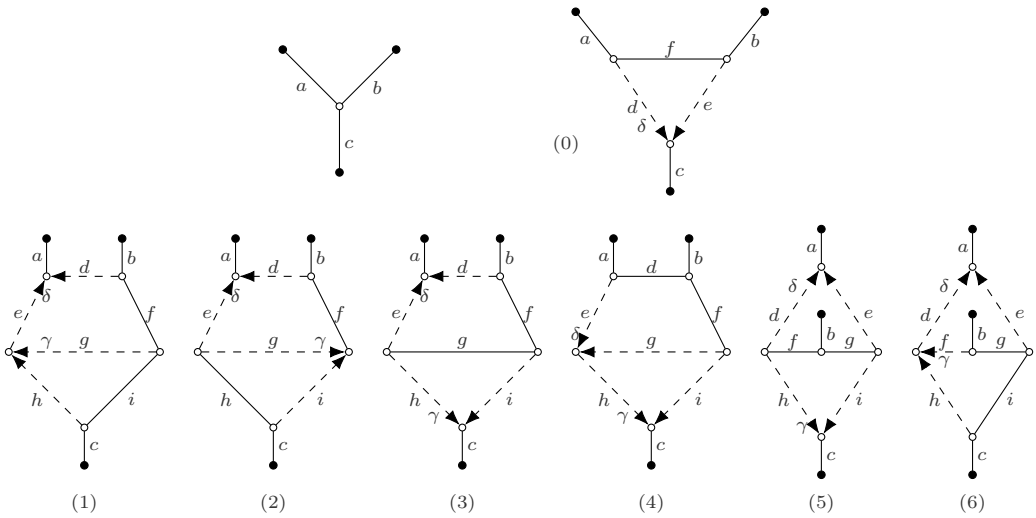

FIGURE 7. All eight simple level-2 trinets up to labeling the leaves. The edge and trinet labels are used in the proof of Proposition 2.15.

First consider the case where N is a 3-leaf tree. Then using the JC Fourier parameters shown in Figure 7, $q_{111} = abc$, $q_{110} = ab$, $q_{101} = ac$, and $q_{011} = bc$, so the expression of (3) evaluates to 0.

Note that if N is not a 3-leaf tree, it can either be the strict level-1 trinet, or one of the six strict level-2 trinets (see Figure 7 for their numbering). We now show that the invariant is strictly positive if N is the trinet of type 0 and defer the very similar calculations for the trinets of types 1-6 to Appendix A.

Trinet 0. Let a-f be the Fourier parameters associated to the edges of N (see also Figure 7). Furthermore, let $\delta \in (0,1)$ be the reticulation parameter. Then, N has the following Fourier parameterizations under the JC model:

$$\begin{aligned} q_{111} &= abcf(\delta d + (1-\delta)e) \\ q_{110} &= abf \\ q_{101} &= ac(\delta d + (1-\delta)ef) \\ q_{011} &= bc(\delta df + (1-\delta)e). \end{aligned}$$

Thus,

$$q_{011}q_{101}q_{110} - q_{111}^2 = a^2b^2c^2f\delta(1-\delta)de(1-f)^2.$$

Since all parameters are in $(0,1)$, $q_{011}q_{101}q_{110} - q_{111}^2$ is strictly positive. □

While we have only been able to find a proof of Proposition 2.15 for level-2 networks, and only by analyzing each network on a case by case basis, we conjecture that the analog of this result should hold for trinets of arbitrary level.

Conjecture 2.16. *Let N be a strict level-k, semi-directed trinet with $k > 0$. Then, under the JC model, the invariant (3) is strictly positive.*

We call a leaf x of a simple quarnet N on S a *prune leaf*, if $N|_{S\setminus\{x\}}$ is a three-leaf tree. As an example, the leaves in the top left of the planar representations in Figure 5 of the quarnets of type 0, 1c, 1d, 1e, 2d, and 3b are prune leaves. As a direct application of Proposition 2.15 and the fact that the quarnets of type 0, 1c, 1d, 1e, 1f, 2d, 3b and 3e have exactly one prune leaf,

and the other quarnets have no prune leaves, we obtain the following useful result, which will be used to prove our main theorem.

Corollary 2.17. *Let N_1 and N_2 be two binary level-2 semi-directed phylogenetic networks on the same leaf set $\mathcal{X}$. Let S be a 4 leaf subset of $\mathcal{X}$. Then, under the JC model:*

(a) if $N_1|_S$ is a type 0, 1c, 1d, 1e, 1f, 2d, 3b or 3e quarnet and $N_2|_S$ is a type 1a, 1b, 2a, 2b, 2c, 3a, 3c or 3d quarnet, then N_1 and N_2 are distinguishable;

(b) if $N_1|_S$ and $N_2|_S$ are distinct quarnets of type 0, 1c, 1d, 1e, 1f, 2d, 3b or 3e with different prune leaves, then N_1 and N_2 are distinguishable.

3. Results

3.1. **Identifiability of Level-1 Networks.** Building on the results of the previous subsections we are now ready to prove the main identifiability result for level-2 networks. Before we do this, we first strengthen the best existing identifiability results for semi-directed level-1 networks under a network-based Markov model from Gross et al. (2021). They proved that the network parameter of a network-based Markov model with respect to the class of n-leaf triangle-free, level-1 semi-directed networks with a fixed number of $r \geq 0$ reticulation nodes is identifiable under the JC, K2P and K3P constraints. Using our previous results, we are able to considerably strengthen these results in the case of JC and K2P constraints. In particular, the following theorem shows that we cannot only identify such level-1 networks if they have the same number of reticulation nodes but also in case the number of reticulation nodes differs. Furthermore, for the JC constraints we can relax the restriction that the networks need to be triangle-free.

Theorem 3.1.

(a) The network parameter of a network-based Markov model under the JC constraints is generically identifiable up to the choice of reticulation nodes in triangles, with respect to the class of models where the network parameter is an n-leaf binary level-1 semi-directed phylogenetic network.

(b) The network parameter of a network-based Markov model under the K2P constraints is generically identifiable with respect to the class of models where the network parameter is an n-leaf binary, triangle-free, level-1 semi-directed phylogenetic network.

Proof. The tree-of-blobs of a network is generically identifiable under both the JC and K2P constraints by Corollary 2.12. By Proposition 2.15, triangles can also be distinguished from trivial blobs under the JC constraints. The results then follow from the fact that simple level-1 networks (or, *sunlets*) with at least four leaves are identifiable under the JC (Gross and Long, 2018) and K2P (Hollering and Sullivant, 2021) constraints. □

3.2. **Identifiability of Level-2 Networks.** Puzzling together distinguishability results from previous sections, Lemmas A.2 and A.3 in Appendix A.4 show distinguishability under the JC constraints of classes of outer-labeled planar and not outer-labeled planar simple level-2 networks, respectively. These lemmas now allow us to prove our main result in the next theorem. Note that Theorem 3.1a is not a special case of this theorem, since here we also assume that the network is triangle-free.

Theorem 3.2. *The network parameter of a network-based Markov model under the Jukes-Cantor constraints is generically identifiable with respect to the class of models where the network parameter is an n-leaf binary, triangle-free, strongly tree-child, level-2 semi-directed phylogenetic network.*

Proof. Let N_1 and N_2 be two distinct n-leaf binary, triangle-free, strongly tree-child, level-2 semi-directed phylogenetic networks on the same leaf set $\mathcal{X}$. If N_1 and N_2 have non-isomorphic trees-of-blobs, they are distinguishable by Corollary 2.12. Since a network is strongly tree-child if and only if the simple networks induced by each blob are strongly tree-child — a fact that follows from Theorem 5 in Holtgrefe et al. (2025b), see also Definition 2.3 — we may consider each blob separately and it will be enough to assume that N_1 and N_2 are both simple networks. Note that N_1 and N_2 cannot contain nontrivial 3-blobs since those are not triangle-free or not strongly tree-child (see Figure 7), so we may also assume N_1 and N_2 have at least 4 leaves.

If N_1 and N_2 are both not outer-labeled planar, then the strongly tree-child assumption forces both networks to be stack-free. Then, they are distinguishable by Lemma A.2. If N_1 and N_2 are both outer-labeled planar, they are distinguishable by Lemma A.3. Lastly, without loss of generality, assume that N_1 is outer-labeled planar and N_2 is not. Then, N_2 is strict level-2 and there is some $S \subseteq \mathcal{X}$ with $|S| = 4$ such that the quarnet $N_2|_S$ is stack-free and not outer-labeled planar and thus is of type 3a, 3b, 3c or 3d. On the other hand, $N_1|_S$ must be outer-labeled planar and can thus not be of type 3a, 3b, 3c or 3d. But the type 3a, 3b, 3c or 3d quarnets are the only level-2 quarnets that display exactly three quartet trees. Therefore, N_1 and N_2 are distinguishable by Theorem 2.11, which lets us distinguish quarnets on the basis of their displayed trees. □

3.3. **Identifiability Beyond Level-2.** In Section 2.2 we proved that semi-directed networks with different displayed quartets are identifiable (Theorem 2.11). Consequently, we also obtained two interesting identifiability results regarding the tree-of-blobs (Corollary 2.12) and the congruent circular orderings of networks (Corollary 2.13), which we later used to obtain our proof of identifiability for level-2 networks. We summarize these three results here in the Results section since they are also of independent interest (see e.g. Allman et al. (2023); Rhodes et al. (2025) for similar results under coalescent based models), especially because the results pertain to networks of any level. Note that these results hold under both the JC and K2P models.

Theorem 3.3. *Let N_1 and N_2 be two binary semi-directed phylogenetic networks on the same leaf set $\mathcal{X}$. Let $\mathcal{M}_1$ and $\mathcal{M}_2$ be the corresponding sets of probability distributions produced by the Markov models on these networks under either the JC or K2P constraints.*

(a) If there is a 4 element subset $S \subseteq \mathcal{X}$ such that the quarnets $N_1|_S$ and $N_2|_S$ have different sets of displayed quartets, then $\mathcal{M}_1 \cap \mathcal{M}_2 = \emptyset$.
(b) If the trees-of-blobs of N_1 and N_2 are not isomorphic, then $\mathcal{M}_1 \cap \mathcal{M}_2 = \emptyset$.
(c) If N_1 and N_2 are both outer-labeled planar and they have different sets of congruent circular orders, then $\mathcal{M}_1 \cap \mathcal{M}_2 = \emptyset$.

4. Discussion

We have shown that the class of strongly tree-child, triangle-free, level-2, binary semi-directed networks is identifiable under the Jukes-Cantor model of evolution. Our results indicate that, under this model, nucleotide sequence data provides enough information to infer semi-directed networks from this class, which can be used as a realistic estimate of a phylogeny from DNA sequence data. This is the first identifiability result of a large class of networks beyond the level-1 framework. As detailed by Kong et al. (2022), a tree-child scenario is more likely to appear in nature than a non-tree-child scenario, since hybridization is considered to be an uncommon evolutionary event, further supporting the biological relevance of our identified class.

The results in this paper do not strictly pertain to level-2 networks. Specifically, we have also shown that the tree-of-blobs of any binary semi-directed network and the circular ordering of the subnetwork around each outer-labeled planar blob are identifiable under both the Jukes-Cantor

and the Kimura-2 parameter models, thereby extending existing results in Allman et al. (2023); Rhodes et al. (2025) to other classes of models. Identifiability of the tree-of-blobs also allowed us to considerably strengthen previous identifiability results for level-1 networks under Markov models (Gross et al., 2021).

It remains an open problem to try to prove identifiability of level-2 (and higher level) networks for broader classes of networks than we have shown in this paper. However, there are already challenges such that for the Markov models it is not possible to identify a network structure in full generality. For example, stacked reticulation nodes always lead to non-identifiability issues, as shown in Sullivant (2025). Even for level-1, it remains open to prove identifiability results for nonbinary networks.

The combinatorial component of our proof, which relies on algebraic distinguishability proofs for small networks, may provide a blueprint to prove similar level-2 identifiability results under other models of evolution. For instance, to lift our level-2 identifiability result to the Kimura 2-parameter model, it would suffice to generalize Lemma 2.14 and Proposition 2.15, which establish distinguishability for specific combinations of small networks. Similarly, the combinatorial part of our proofs could be useful when attempting to generalize our results to settings with incomplete lineage sorting. Going beyond the level-2 assumption will likely require further improvements to the existing algebraic tools. Notably, it is already known that semi-directed level-3 networks are not encoded by their quarnets (Huber et al., 2025). As a result, achieving identifiability of (a subclass of) level-3 networks may necessitate analyzing numerous combinations of induced level-3 quinnets (5-leaf networks), leading to a combinatorial explosion of combinations compared to level-2 quarnets.

On the algebraic side, there are many open questions related to level-2 networks, and even quarnets. For example, while we computed some phylogenetic invariants for these networks in some cases, in most cases it remains open to try to determine the vanishing ideal of the model, or other basic algebraic properties of the models. We found instances of pairs of networks that have the same underlying algebraic matroid but different vanishing ideals. There were examples of networks that had a lower dimension than would be expected based on a simple parameter count. Developing tools to explain these phenomena leads to interesting algebraic challenges, and may provide new tools for proving deeper identifiability results.

Finally, on the algorithmic side, our findings open several promising research directions. Previous identifiability proofs for level-1 networks (Gross et al., 2021) have resulted in theoretical algorithms that puzzle together quarnets to construct a complete level-1 network (Huebler et al., 2025; Frohn et al., 2025), ultimately contributing to a quarnet-based practical tool for inference of level-1 networks (Holtgrefe et al., 2025a). Building on our results, similar theoretical algorithms could serve as a foundation for consistent level-2 network inference methods. However, such algorithms, which rely on assembling multiple quarnets, also require consistent methods for inferring these quarnets from biological data. Two existing approaches, both based on invariants, have been developed for level-1 quarnets (Barton et al., 2022; Martin et al., 2023). To establish a complete pipeline from sequence data to level-2 network reconstruction, analogous techniques may be necessary for level-2 quarnets.

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Data Availability Statement

The `Macaulay2` scripts necessary to reproduce the proof of Lemma 2.14 and the `Maple` scripts used to verify the proof of Proposition 2.15 can be accessed at: http://datadryad.org/share/hlcT6lnstcCbZ5M_oaqLHRM7CJ-5sRWOE8IGV7st7kc.

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Appendix A. Omitted proofs

Our main result relies on a mixture of algebraic, computational and combinatorial results, with several of the longer proofs presented in this appendix.

A.1. Distinguishing Networks with Different Displayed Quartets.

Proposition 2.10. *Consider the Fourier coordinates q associated to a quartet tree T with leaf set $\{1,2,3,4\}$ under the JC or K2P model. Then, the invariant*

$$q_{GGGG} - q_{GGTT} - q_{GTTG} + q_{GTGT} \tag{2}$$

evaluates to zero if T has split $12|34$ or $14|23$ and is strictly positive if T has split $13|24$.

Proof. Under the tree $12|34$ we have

$$\begin{aligned} q_{GGGG} &= a_G^1 a_G^2 a_G^3 a_G^4 a_A^{12|34} \\ q_{GGTT} &= a_G^1 a_G^2 a_T^3 a_T^4 a_A^{12|34} \\ q_{GTTG} &= a_G^1 a_T^2 a_T^3 a_G^4 a_C^{12|34} \\ q_{GTGT} &= a_G^1 a_T^2 a_G^3 a_T^4 a_C^{12|34}. \end{aligned}$$

Since $a_G^e = a_T^e$ for all edges e, we see that $q_{GGGG} = q_{GGTT}$ and $q_{GTTG} = q_{GTGT}$, so expression 2 evaluates to 0.

Similarly, under the tree $14|23$ we have

$$\begin{aligned} q_{GGGG} &= a_G^1 a_G^2 a_G^3 a_G^4 a_A^{14|23} \\ q_{GGTT} &= a_G^1 a_G^2 a_T^3 a_T^4 a_C^{14|23} \\ q_{GTTG} &= a_G^1 a_T^2 a_T^3 a_G^4 a_A^{14|23} \\ q_{GTGT} &= a_G^1 a_T^2 a_G^3 a_T^4 a_C^{14|23}, \end{aligned}$$

so $q_{GGGG} = q_{GTTG}$ and $q_{GGTT} = q_{GTGT}$. Thus, expression 2 again evaluates to 0.

On the other hand, under the tree $13|24$ we have that

$$\begin{aligned} q_{GGGG} &= a_G^1 a_G^2 a_G^3 a_G^4 a_A^{13|24} \\ q_{GGTT} &= a_G^1 a_G^2 a_T^3 a_T^4 a_C^{13|24} \\ q_{GTTG} &= a_G^1 a_T^2 a_T^3 a_G^4 a_C^{13|24} \\ q_{GTGT} &= a_G^1 a_T^2 a_G^3 a_T^4 a_A^{13|24}. \end{aligned}$$

So, expression 2 becomes $2a_G^1 a_G^2 a_G^3 a_G^4 (1 - a_C^{13|24})$ since $a_G^e = a_T^e$ and $a_A^e = 1$ for all edges e. Because $a_C^{13|24} \in (0,1)$, this shows that expression 2 is positive. □

The following result is presented in a slightly different form in Rhodes et al. (2025). Since the original source presents only an implicit proof in a different context (Allman et al., 2023), we prove part (a) here as well.

Proposition A.1. *Let N be a binary semi-directed phylogenetic network on 4 leaves $\{a, b, c, d\}$. Then,*

(a) N induces the nontrivial split $ab|cd$ if and only if N displays solely the quartet with nontrivial split $ab|cd$;

(b) if N is simple and outer-labeled planar (congruent with circular order (a, b, c, d)), then N displays exactly the two quartets with nontrivial splits $ab|cd$ and $ad|bc$.

Proof of part (a). The only-if direction of part (a) is trivial since every quartet displayed by N has the cut-edge induced by the split $ab|cd$ in its embedding. Thus, all the quartets displayed by N have the split $ab|cd$.

For the if-direction of part (a), we use induction on the level k of the network. Assume that N does not induce any nontrivial split. For $k = 0$ this is not possible and for $k = 1$, N will contain a 4-cycle, causing it to display two quartets with different splits, so we are done. For $k \geq 2$, pick a reticulation r with a leaf x below it. Create two level-$(k-1)$ networks N_1, N_2 by deleting one of the two reticulation edges entering r, undirecting the other reticulation edge and exhaustively deleting reticulation nodes with in-degree 2 and out-degree 0, suppressing non-reticulate degree-2 nodes and suppressing parallel edges. If at least one of N_1, N_2 does not induce a nontrivial split, then it displays at least two quartets by induction. Hence, N also displays at least two quartets. Now consider the remaining case, that both N_1 and N_2 induce a nontrivial split. Then it is sufficient to show that N_1 and N_2 display different nontrivial splits.

Suppose that N_1 and N_2 display the same nontrivial split, say $xy|wz$. Let N' be the mixed graph obtained from N by deleting x and exhaustively deleting resulting reticulation nodes with in-degree 2 and out-degree 0. Note that N' is connected since otherwise N could not be oriented as a directed phylogenetic network. Let e be the cut-edge of N' inducing the split $xy|wz$ with maximum distance from y. Let N'' be the connected component of the mixed graph obtained from N' by deleting e, such that N'' contains w and z. Since N does not have a cut-edge inducing the split $xy|wz$, it contains a directed path from N'' to the neighbour of x, which does not use e. Hence, N_i contains a directed path from N'' to the neighbour of x for some $i \in \{1, 2\}$ that does not use e. However, this implies that N_i does not have a cut-edge inducing the split $xy|wz$, a contradiction. □

A.2. **Distinguishing Small Networks using Invariants and Matroids.**

Lemma 2.14. *Let N_1 and N_2 be two binary level-2 semi-directed phylogenetic networks on the same leaf set $\mathcal{X}$. Let S be a 4 leaf subset of $\mathcal{X}$. Then, under the JC model:*

(a) if $N_1|_S$ and $N_2|_S$ are distinct quarnets of type 1b with a different leaf in the top middle in the planar representation of Figure 5, then N_1 and N_2 are distinguishable;

(b) if $N_1|_S$ and $N_2|_S$ are distinct quarnets of type 2a in Figure 5, then N_1 and N_2 are distinguishable;

(c) if $N_1|_S$ and $N_2|_S$ are distinct quarnets of type 3a, 3c or 3d in Figure 5, then N_1 and N_2 are distinguishable.

Let $S = \{a, b, c, d, e\}$ be a 5 leaf subset of X. Then, under the JC model:

(d) if $N_1|_S$ and $N_2|_S$ are the two quinnets from Figure 6, then N_1 and N_2 are distinguishable.

Remainder of the proof. **(a)** This computation is verified in the file `Lemma214a.m2`. First of all, we only need to look at the different labelings of the leaves of quarnet 1b that have the same displayed trees. With the leaves labeled clockwise starting in the upper left, this amounts to looking at the eight circular orders

$$1234, 2341, 3412, 4123, 4321, 1432, 2143, 3214.$$

Here we want to show that each of these circular orders is distinguished from the others except in the case that the top middle leaf gets the same label, e.g. we do not need to distinguish the pair $1234, 3214$. In fact, separate computations show that those pairs have the same ideal. As explained below Proposition 2.6, this does not prove that the corresponding models are indistinguishable, although it does provide evidence in favor of this conclusion.

For each of the pairs we want to distinguish, there is a subset of the variables that has the property that the corresponding columns of the Jacobian matrices under the networks have different ranks. These subsets are listed in "successList" in the file. For example, for the first pair (corresponding to the two orders $1234, 2341$, the set of columns of the Jacobian matrix

$$\{0, 1, 3, 4, 5, 6, 7, 9, 10\}$$

yield different ranks in the two networks. These correspond to the indeterminates

$$q_{AAAA}, q_{AACC}, q_{ACAC}, q_{ACGT}, q_{CACA}, q_{CAAC}, q_{CAGT}, q_{CGTA}, q_{CGTA}.$$

Running the code and producing the term "listofrankpairs" shows the ranks of the corresponding submatrices of the Jacobians for these two networks. It has rank 8 for ordering 1234 and rank 9 for ordering 2341.

(b) The proof of (b) follows a similar strategy as (a), and the corresponding computation is in the file `Lemma214b.m2`.

(c) The proof of (c) is established by finding phylogenetic invariants that distinguish each pair of networks. In the file `InvariantForThreeA.m2`, we give a degree 6 invariant with 53 terms that distinguishes quarnet 3a with the labeling 1234 from all other labeled 3a, 3b, and 3d quarnets. In the file `InvariantForThreeC.m2`, we give a degree 5 invariant with 34 terms that distinguishes the 3c quarnet with the labeling 1234 from all other labeled 3a, 3b, and 3d quarnets, and in the file `InvariantForThreeD.m2`, we give a degree 5 invariant with 50 terms that distinguishes the 3d quarnet with labeling 1234 from all other labeled 3a, 3b, and 3d quarnets. The computation verifying the statements is in the file `Lemma214c.m2`.

(d) The proof of (d) follows a similar strategy as (a), and the corresponding computation is in the file `Lemma214d.m2`. In this case there is just one pair of networks to compare. Considering the set of indeterminates

$$q_{AACCA}, q_{ACAAC}, q_{ACACA}, q_{ACCAA}, q_{CACAA}, q_{ACAGT},$$

$$q_{ACGTA}, q_{CAGAT}, q_{CAGTA}, q_{CGAAT}, q_{CGATA}, q_{CGTAA}$$

the code shows that for the left network, the corresponding rank of the submatrix of the Jacobian is 12, and for the right network it is 11. □

A.3. **Distinguishing Three-Leaf Trees from Trinets.**

Proposition 2.15. *Consider the Fourier coordinates q associated to a binary, semi-directed level-2 trinet N under the JC model. Then the invariant*

$$q_{011}q_{101}q_{110} - q_{111}^2 \tag{3}$$

evaluates to zero if N is a three-leaf tree and is strictly positive if N is a strict level-1 or strict level-2 trinet.

Remainder of the proof. In the main text we showed that the invariant (3) evaluates to zero for the three-leaf tree, and that it is positive on the trinet of type 0 (see Figure 7 for the trinet numbering), assuming the parameter values are in $(0, 1)$. Here, we complete the proof by providing the Fourier parameterizations of the trinets 1-6 under the JC model (using the parameters as given in Figure 7) and proving positivity of expression (3) by providing a suitable decomposition. Correctness of the decompositions can be verified using the `Maple` files `trinet1.mw` – `trinet6.mw`.

Trinet 1. As depicted in Figure 7, let a-i be the nontrivial Fourier parameters associated to the edges of N. Furthermore, let δ and γ be the two reticulation parameters. Under the JC model, N has the following Fourier parameterizations:

$$\begin{aligned}
q_{111} &= abcfi(\delta e(\gamma g + (1-\gamma)h) + (1-\delta)d) \\
q_{110} &= ab(\delta ef(\gamma g + (1-\gamma)hi) + (1-\delta)d) \\
q_{101} &= ac(\delta e(\gamma gi + (1-\gamma)h) + (1-\delta)dfi) \\
q_{011} &= bcfi.
\end{aligned}$$

We now compute $q_{110}q_{101}q_{011} - q_{111}^2$ and look at the coefficients for δ^2, $(1-\delta)^2$ and $\delta(1-\delta)$. The coefficient for δ^2 is

$$a^2b^2c^2e^2f^2ghi \cdot \gamma(1-\gamma)(1-i)^2.$$

The coefficient for $(1-\delta)^2$ is as follows:

$$a^2b^2c^2\Big(d^2f^2i^2 - d^2f^2i^2\Big) = 0.$$

Lastly, the coefficient for $(1-\delta)\delta$ becomes

$$a^2b^2c^2defi\Big(\gamma gi(1-f)^2 + (1-fi)^2(1-\gamma)h\Big).$$

Since the parameter values are in $(0, 1)$, the coefficients for δ^2 and $(1-\delta)\delta$ are positive, while the coefficient for $(1-\delta)^2$ was equal to zero. All in all, this shows that the invariant 3 is positive.

Trinet 2. The Fourier parameterizations of Trinet 2 are as follows:

$$\begin{aligned}
q_{111} &= abc(\delta efh(\gamma g + (1-\gamma)i) + (1-\delta)df(\gamma gh + (1-\gamma)i)) \\
q_{110} &= ab(\delta ef(\gamma g + (1-\gamma)hi) + (1-\delta)d) \\
q_{101} &= ac(\delta eh + (1-\delta)df(\gamma gh + (1-\gamma)i)) \\
q_{011} &= bcf(\gamma gh + (1-\gamma)i).
\end{aligned}$$

For the invariant we get that

$$\begin{aligned} q_{011}q_{101}q_{110} - q_{111}^2 &= \delta^2 \cdot a^2b^2c^2e^2f^2\,\gamma(1-\gamma)\,ghi\,(1-h)^2 \\ &\quad + \delta(1-\delta)\cdot a^2b^2c^2de\,(\gamma fgh + fi(1-\gamma))\cdot \\ &\quad \Big(h(1-f(\gamma g+(1-\gamma)i))^2 + f^2g(1-h)^2 i\gamma(1-\gamma)\Big). \end{aligned}$$

which is positive since all parameter values are in $(0,1)$.

Trinet 3. The Fourier parameterizations for Trinet 3 are

$$\begin{aligned} q_{111} &= abc(\delta efg(\gamma h+(1-\gamma)i) + (1-\delta)df(\gamma gh+(1-\gamma)i)) \\ q_{110} &= ab(\delta efg + (1-\delta)d) \\ q_{101} &= ac(\delta e(\gamma h+(1-\gamma)gi) + (1-\delta)df(\gamma gh+(1-\gamma)i)) \\ q_{011} &= bcf(\gamma gh+(1-\gamma)i). \end{aligned}$$

Then, one obtains that

$$\begin{aligned} q_{011}q_{101}q_{110} - q_{111}^2 &= \delta^2 \cdot a^2b^2c^2e^2f^2ghi(1-g)^2\gamma(1-\gamma) \\ &\quad + \delta(1-\delta)\cdot a^2b^2c^2def(gh\gamma + i(1-\gamma))\cdot \\ &\quad \Big(h(1-fg)^2\gamma + gi(1-f)^2(1-\gamma)\Big), \end{aligned}$$

which is again positive since all parameter values are in $(0,1)$.

Trinet 4. The Fourier coordinates of Trinet 4 are:

$$\begin{aligned} q_{111} &= abc(\gamma dh(\delta e+(1-\delta)fg) + (1-\gamma)dfi) \\ q_{110} &= abd \\ q_{101} &= ac(\gamma h(\delta e+(1-\delta)dfg) + (1-\gamma)dfi) \\ q_{011} &= bc(\gamma h(\delta de+(1-\delta)fg) + (1-\gamma)fi). \end{aligned}$$

The invariant for Trinet 4 is

$$\begin{aligned} q_{011}q_{101}q_{110} - q_{111}^2 &= \gamma^2 \cdot a^2b^2c^2defgh^2\delta(1-\delta)(1-d)^2 \\ &\quad + (1-\gamma)\gamma\cdot a^2b^2c^2d(1-d)^2efhi\delta, \end{aligned}$$

which is positive under our parameter constraints.

Trinet 5. The Fourier coordinates are now as follows:

$$\begin{aligned} q_{111} &= abc(\delta df(\gamma h+(1-\gamma)ig) + (1-\delta)eg(\gamma hf+(1-\gamma)i)) \\ q_{110} &= ab(\delta df + (1-\delta)eg) \\ q_{101} &= ac(\delta d(\gamma h+(1-\gamma)ifg) + (1-\delta)e(\gamma hfg+(1-\gamma)i)) \\ q_{011} &= bc(\gamma fh + (1-\gamma)gi). \end{aligned}$$

The invariant then becomes

$$\begin{aligned} q_{011}q_{101}q_{110} - q_{111}^2 &= \delta^2 \cdot (abcd)^2fghi\gamma(1-\gamma)(f-1)^2 \\ &\quad + (1-\delta)^2\cdot(abce)^2fghi\gamma(1-\gamma)(g-1)^2 \\ &\quad + (1-\delta)\delta\cdot\Big((abc)^2de(\gamma^2fgh^2(f-1)^2 \\ &\quad + (1-\gamma)^2fgi^2(g-1)^2 + (1-\gamma)\gamma hi(f-g)^2)\Big). \end{aligned}$$

This can again be seen to be positive since all parameter values are in $(0,1)$.

Trinet 6. The Fourier parameterizations of Trinet 6 are

$$\begin{aligned} q_{111} &= abc(\delta dgi(\gamma f + (1-\gamma)h) + (1-\delta)egi) \\ q_{110} &= ab(\delta d(\gamma f + (1-\gamma)ghi) + (1-\delta)eg) \\ q_{101} &= ac(\delta d(\gamma fgi + (1-\gamma)h) + (1-\delta)ei) \\ q_{011} &= bcgi. \end{aligned}$$

After simplification, the invariant becomes

$$\begin{aligned} q_{011}q_{101}q_{110} - q_{111}^2 &= \delta^2 \cdot a^2b^2c^2d^2fghi\gamma(1-\gamma)(1-gi)^2 \\ &\quad + (1-\delta)\delta \cdot a^2b^2c^2degi\left((gh(i-1)^2(1-\gamma) + fi\gamma(g-1)^2)\right) \end{aligned}$$

This is positive provided all parameters are positive and smaller than 1. □

A.4. **Distinguishing Large Level-2 Networks.** This section focuses on distinguishing certain pairs of n-leaf, simple, semi-directed, level-2 networks under the JC model. We state our results in the form of two key lemmas that rely on our previous results to argue that any pair of considered networks has a subnetwork that distinguishes them. We implicitly use Proposition 2.8 to then extend the distinguishability to the full network.

Lemma A.2. *Let N_1 and N_2 be distinct binary, stack-free, simple, semi-directed level-2 phylogenetic networks on the same leaf set. If neither N_1 nor N_2 is outer-labeled planar, then N_1 and N_2 are distinguishable under the JC constraints.*

Proof. Let $\mathcal{X}$ be the leaf set of the two networks. First note that both N_1 and N_2 are strict level-2, otherwise they would be outer-labeled planar. The only strict level-2 quarnets that are stack-free and not outer-labeled planar are of type 3a, 3c or 3d (see Figure 5 for the quarnet

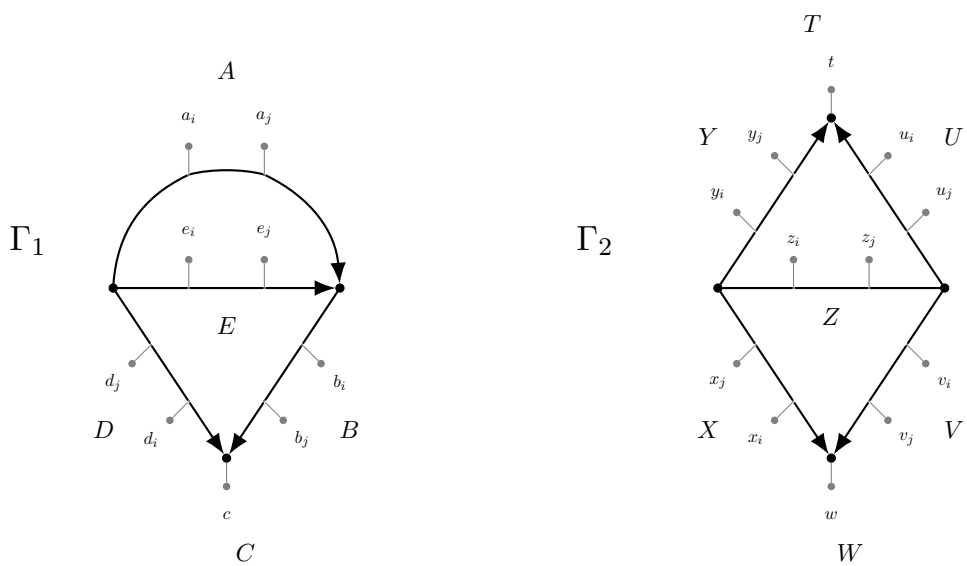

FIGURE 8. The two generators Γ_1 and Γ_2 (in thick black) of a simple, strict level-2, semi-directed network (Huber et al., 2025, Lem. 4.2). The sides $\{A, B, C, D, E\}$ and $\{T, U, V, W, X, Y, Z\}$ of the two generators are the places where the leaves (in gray) might be attached in the corresponding semi-directed network.

numbering). Hence, if $|\mathcal{X}| = 4$, N_1 and N_2 are distinguishable by Lemma 2.14c and we may thus assume that $|\mathcal{X}| \geq 5$.

Let G be the *generator* of N_1, i.e., the mixed multi-graph obtained from N_1 by deleting all leaves and suppressing degree-2 nodes. Similarly, we let H be the generator of N_2. By Lemma 4.2 in Huber et al. (2025), there are exactly two possibilities for G and H, depicted in Figure 8: the generators Γ_1 and Γ_2. The edges and reticulation nodes with in-degree 2 and out-degree 0 of Γ_1 and Γ_2 are called *sides*. For a side S of Γ_1 or Γ_2, we call a leaf s *on side* S if leaf s is attached to side S in the original network. We now consider three cases. We will implicitly use that for every $S \subseteq \mathcal{X}$ with $|S| = 4$ the two quarnets $N_1|_S$ and $N_2|_S$ either both have the same nontrivial split or neither has a nontrivial split, otherwise the two quarnets have a distinct tree-of-blobs and the networks are already distinguishable by Corollary 2.12.

Case 1: $G \ncong H$. Without loss of generality we assume that $G \cong \Gamma_1$ and $H \cong \Gamma_2$. In N_1, let a be any leaf on side A, b any leaf on side B, c the leaf on side C, and e any leaf on side E. Note that a and e exist since N_1 is not outer-labeled planar, and that b exists since N_1 is stack-free. Then, the quarnet $N_1|_S$ with $S = \{a, b, c, e\}$ is of type 3a. Now observe that quarnets of type 3a, 3b and 3e each contain one reticulation node whose child is not a leaf. Since only leaves can be added to sides T and W in Γ_2, this means $N_2|_S$ cannot be of type 3a, 3b or 3e. Thus, $N_2|_S$ is distinct from $N_1|_S$. Specifically, it is of type 0, 1, 2, 3c or 3d. Then, if $N_2|_S$ is of type 0, 1 or 2, N_1 and N_2 are distinguishable by Theorem 2.11, since $N_2|_S$ then displays at most two quartet trees, whereas $N_1|_S$ displays three quartet trees. Otherwise, if $N_2|_S$ is a quarnet of type 3c or 3d, N_1 and N_2 are distinguishable by Lemma 2.14c.

Case 2: $G \cong H \cong \Gamma_1$. Let $S = \{a, b, c, e\}$ be an arbitrary set of leaves as in Case 1, such that $N_1|_S$ is a quarnet of type 3a. Similar reasoning as in Case 1 then shows that N_1 and N_2 are distinguishable if for some choice of S the quarnet $N_1|_S$ is distinct from $N_2|_S$. Hence, we may assume that $N_1|_S$ and $N_2|_S$ are isomorphic for any valid choice of S. It follows that N_1 and N_2 share the same leaves on the sides A, B, C and E of their generator (modulo swapping sides A and E), and therefore also on side D. Then, since by assumption N_1 and N_2 are distinct and $|\mathcal{X}| \geq 5$, we have that for some side $\Sigma \in \{A, B, D, E\}$ (with $|\Sigma| \geq 2$) two leaves $\sigma_i \in \Sigma$ and $\sigma_j \in \Sigma$ are ordered differently in the two networks. Now, let c be the leaf on side C of N_1 and N_2. First suppose that $\Sigma \in \{A, D, E\}$ and let b be a leaf on side B of N_1 and N_2 (which exists since the networks are stack-free). Then, the quarnets $N_1|_S$ and $N_2|_S$ with $S = \{\sigma_i, \sigma_j, b, c\}$ are both simple and outer-labeled planar, i.e. both are congruent with a single circular ordering of S. But since σ_i and σ_j are ordered differently on side Σ in the two networks, these circular orders cannot be the same. Hence, N_1 and N_2 are distinguishable by Corollary 2.13, which states that two simple and outer-labeled planar quarnets are distinguishable if they are not congruent with the same circular ordering. The case where $\Sigma = B$ follows a similar argument, instead considering the subset of leaves $S = \{\sigma_i, \sigma_j, a, c\}$, where a is a leaf from side A of both networks (which exists since the networks are not outer-labeled planar).

Case 3: $G \cong H \cong \Gamma_2$. In N_1, let t be the leaf on side T, w the leaf on side W, z any leaf on side Z, and σ any other leaf. Note that t, w and z exist since N_1 is not outer-labeled planar. Then, the quarnet $N_1|_S$ with $S = \{t, w, z, \sigma\}$ is of type 3c or 3d. If for some valid choice of S the quarnet $N_2|_S$ is of type 0, 1 or 2, we get that N_1 and N_2 are distinguishable by Theorem 2.11, since $N_2|_S$ then displays at most two quartet trees, whereas $N_1|_S$ displays three quartet trees.. Now note that if $N_2|_S$ is not of type 0, 1 or 2, it can only be of type 3c or 3d. By Lemma 2.14c, two distinct quarnets of type 3c or 3d are distinguishable, so we may now assume that $N_1|_S$ and $N_2|_S$ are isomorphic for all valid choices of S. It follows that N_1 and N_2 share the same leaves on the sides T, $Y \cup U$, Z, $X \cup V$ and W of their generators (modulo swapping $\{Y, T, U\}$ with $\{X, W, V\}$).

If $|Y \cup U \cup X \cup V| \leq 1$ for N_1 and N_2, then N_1 and N_2 share the same leaves on every side (modulo swapping Y with U and swapping X with V). If $|Y \cup U \cup X \cup V| \geq 2$ for N_1 and N_2, let σ and π be two arbitrary leaves in this set. Furthermore, let t be the leaf on side T of N_1 and N_2 and w the leaf on side W of N_1 and N_2. Then, $N_1|_S$ and $N_2|_S$ with $S = \{\sigma, \pi, t, w\}$ are both simple and outer-labeled planar quarnets, i.e. both congruent with a single circular order of S. If for some valid choice of S the induced orderings are not the same, then N_1 and N_2 are distinguishable by Corollary 2.13. Thus, we may assume that these circular orders are the same for any such set S, again implying that N_1 and N_2 share the same leaves on every side (modulo swapping Y with U and swapping X with V). Furthermore, the leaves on these four sides must be ordered the same.

The only remaining way for N_1 and N_2 to be distinct is then that there are two leaves z_i and z_j on side Z of N_1 and N_2 that are not ordered the same in the two networks. Let σ be a leaf unequal to z_i or z_j from some side $\Sigma \in \{U, V, X, Y, Z\}$ (which exists since $|\mathcal{X}| \geq 5$). If $\Sigma \in \{U, Y, Z\}$ then we consider the set $S = \{\sigma, t, z_i, z_j\}$ with t the leaf on side T of both networks. Otherwise, if $\Sigma \in \{V, X\}$, we instead pick the other leaf below the reticulation w (on side W of both networks) and consider the set $S = \{\sigma, w, z_i, z_j\}$. In both cases, the quarnets $N_1|_S$ and $N_2|_S$ are of type 0 (i.e. they are level-1 quarnets with a 4-cycle) and they induce a unique circular ordering of S. But since z_i and z_j are ordered differently on side Z in the two networks, these induced orders cannot be the same. Hence, N_1 and N_2 are distinguishable by Corollary 2.13. □

Lemma A.3. *Let N_1 and N_2 be distinct binary, triangle-free, strongly tree-child, simple, semi-directed level-2 phylogenetic networks on the same leaf set. If N_1 and N_2 are both outer-labeled planar, then N_1 and N_2 are distinguishable under the JC constraints.*

Proof. Let $\mathcal{X}$ be the leaf set of the two networks. If N_1 and N_2 are both level-1 networks, they are distinguishable by Gross and Long (2018). If exactly one of them (say N_1) is a strict level-2 network, then there will be some $S \subseteq \mathcal{X}$ with $|S| = 4$ such that $N_1|_S$ is a type 1b or 2a quarnet, since N_1 is triangle-free, strongly tree-child and outer-labeled planar (see Figure 5 for the quarnet numbering). Because N_2 is level-1, $N_2|_S$ is either a type 0 quarnet or a quarnet inducing a nontrivial split. In the first case, $N_1|_S$ and $N_2|_S$ are distinguishable by Corollary 2.17a, whereas in the second case the two quarnets have a distinct tree-of-blobs and are distinguishable by Corollary 2.12. Thus, we may assume that N_1 and N_2 are both strict level-2. Now note that the only outer-labeled planar strict level-2 quarnets that are strongly tree-child and triangle-free are of type 2a. Hence, if $|\mathcal{X}| = 4$, the two networks are distinguishable by Lemma 2.14b. Thus, we will also assume that $|\mathcal{X}| \geq 5$.

As in the proof of Lemma A.2, we let G be the generator of N_1 and H the generator of N_2. See again Figure 8 for the two possible generators Γ_1 and Γ_2. Since N_1 and N_2 are outer-labeled planar, we may assume in any case that the no leaves are attached to side E of Γ_1 and to side Z of Γ_2. We again consider three cases. Note that no quarnets of N_1 or N_2 can be of type 3, since both networks are outer-labeled planar. As in the proof of the previous lemma, we will implicitly use that for every $S \subseteq \mathcal{X}$ the two quarnets $N_1|_S$ and $N_2|_S$ with $|S| = 4$, either both have the same nontrivial split or neither has a nontrivial split, otherwise the two quarnets have a distinct tree-of-blobs and the networks are already distinguishable by Corollary 2.12. Furthermore, by Corollary 2.13, we can assume that N_1 and N_2 share the same circular ordering of their leaf set $\mathcal{X}$, otherwise the two networks are again distinguishable.

Case 1: $G \ncong H$. Without loss of generality we assume that $G \cong \Gamma_1$ and $H \cong \Gamma_2$. In N_1, let $\{a_i, a_j\}$ be any pair of leaves on side A, c the leaf on side C and d any leaf on side D. Note that a_i and a_j exist since N_1 is triangle-free and that d exists since N_1 is strongly tree-child. Then,

the quarnet $N_1|_S$ with $S = \{a_i, a_j, c, d\}$ is of type 2d with c being its prune leaf (see the definition before Corollary 2.17). If $N_2|_S$ is of type 1a, 1b, 2a, 2b or 2c, the two quarnets are distinguishable by Corollary 2.17a, whereas the two quarnets are distinguishable by Corollary 2.17b if $N_2|_S$ is of type 0, 1c, 1d, 1e, 1f or 2d with a prune leaf $\sigma \neq c$. It follows that we may assume that $N_2|_S$ is a quarnet of type 0, 1c, 1d, 1e, 1f or 2d with prune leaf c. This means that c is below a reticulation in $N_2|_S$, and therefore, c has to be on side T or W of N_2.

Let $\{a_i, a_j, d\}$ be as before and pick b as an arbitrary leaf on side B of N_1 (which exists since N_1 is stack-free). Then, the quarnet $N_1|_S$ with $S = \{a_i, a_j, b, d\}$ is of type 0 with the leaf b below the reticulation, i.e. b is its prune leaf. A similar argument as before then shows distinguishability, unless b is on side T or W of N_2. We now show that this is impossible by deriving a contradiction, which will then prove Case 1. If b is on side T or W of N_2, we can assume that the leaves on sides $B \cup C$ of N_1 coincide with the leaves on sides $T \cup W$ of N_2 (which also implies that $|B| = 1$ for N_1). Now note that the leaves b and c are neighbors in the circular ordering of $\mathcal{X}$ induced by N_1. By our assumption, this is then also the case for N_2 (where b and c are on sides T and W). Therefore, the sides X and Y (or U and V) of N_2 are both empty. But then N_2 is not strongly tree-child: a contradiction.

Case 2: $G \cong H \cong \Gamma_1$. In N_1, let $\{a_i, a_j\}$ be any pair of leaves on side A, d any leaf on side D, and σ any leaf on side $\Sigma \in \{B, C\}$. Note that a_i and a_j exist since N_1 is triangle-free and that d exists since N_1 is strongly tree-child. The quarnet $N_1|_S$ with $S = \{a_i, a_j, d, \sigma\}$ is then of type 0 or 2d, in both cases with the leaf σ below the reticulation (i.e. with σ its prune leaf). Similar reasoning (based on Corollary 2.17) as in the previous case then shows that N_1 and N_2 are distinguishable, unless $N_2|_S$ is a quarnet of type 0, 1c, 1d, 1e or 1f with prune leaf σ. Thus, from now on we may assume that for any valid choice of σ, the quarnet $N_2|_S$ has σ below a reticulation, meaning that σ is also on sides $B \cup C$ of N_2. Hence, N_1 and N_2 share the same leaves on sides $B \cup C$ (and therefore also on sides $A \cup D$).

Now, in N_1, let a be a leaf on side A, b a leaf on side B, c the leaf on side C and d a leaf on side D. Note that these leaves all exist since N_1 is strongly tree-child. Furthermore, pick these four leaves such that $\{a, b\}$ and $\{c, d\}$ are both neighboring pairs of leaves in the circular ordering of X induced by N_1 (and hence also by N_2). The quarnet $N_1|_S$ with $S = \{a, b, c, d\}$ is now of type 1b, with c the leaf in the top middle in the planar representation of Figure 5. Recall that N_1 and N_2 share the same circular ordering of their leaf set X and that they share the same leaves on sides $B \cup C$ and $A \cup D$. By our specific choice of the leaves in S, we then have that $N_2|_S$ is either isomorphic to $N_1|_S$, or it is a type 1b quarnet with the same circular ordering as $N_1|_S$ but with the leaf b in the top middle in the planar representation of Figure 5 instead. In the latter case, N_1 and N_2 are distinguishable by Lemma 2.14a. Hence, we can assume that $N_1|_S$ and $N_2|_S$ are isomorphic, also implying that N_1 and N_2 share the same leaf on side C (and therefore also have the same leaves on side B).

Lastly, let c be the leaf on side C of N_1 and N_2. Now consider the trees-of-blobs of the networks $N_1|_S$ and $N_2|_S$ with $S = \mathcal{X} \setminus \{c\}$. First, assume towards a contradiction that these two trees-of-blobs are isomorphic. Then, since the two networks already share the same leaves on their side B and both networks have at least one leaf on their side D, they must share the same number of leaves on their side D (and therefore also on their side A) for the trees-of-blobs to be isomorphic. However, we already know that N_1 and N_2 share the same circular ordering of their leaf set $\mathcal{X}$, showing that N_1 and N_2 share the same leaves on every side and that they are also ordered the same. Therefore, N_1 and N_2 are isomorphic: a contradiction. Thus, we may instead assume that the two trees-of-blobs are not isomorphic. But then $N_1|_S$ and $N_2|_S$ (and therefore also the two networks N_1 and N_2) are distinguishable by Corollary 2.12.

Case 3: $G \cong H \cong \Gamma_2$. Let t be the leaf on side T of N_1 and let w be the leaf on side W of N_1. Note that t and w cannot be neighbors in the circular ordering of N_1 and N_2, otherwise N_1 would not be strongly tree-child. Now consider the set $S = \mathcal{X} \setminus \{t, w\}$. Then, $N_1|_S$ is a tree and all its induced trinets are triplet trees. If this is not the case for $N_2|_S$, the two networks are distinguishable by Proposition 2.15, which can be used to distinguish triplet trees from trinets with a reticulation. Thus, we can assume that $N_2|_S$ also induces only triplet trees, i.e. $N_2|_S$ is a tree. We now show that t and w are then also the leaves on sides T and W of N_2. In particular, if neither t nor w is on sides $T \cup W$ of N_2, we clearly have that $N_2|_S$ is not a tree: a contradiction. On the other hand, assume w.l.o.g. that t is on side T of N_2 and w is not on side W of N_2. Then, since t and w are not neighbors in the circular ordering of $\mathcal{X}$ induced by N_2 and since N_2 is strongly tree-child, there will be some $\sigma_i \notin \{t, w\}$ on sides $X \cup Y$ and some $\sigma_j \notin \{t, w\}$ on sides $U \cup V$ of N_2. Let $w' \notin \{t, w, \sigma_i, \sigma_j\}$ then be the leaf on side W of N_2. The trinet $N_2|_{\{\sigma_i, \sigma_j, w'\}}$ with $\{\sigma_i, \sigma_j, w'\} \subseteq A$ will then not be a triplet tree: a contradiction. Thus, t and w are on sides $T \cup W$ of N_2. Therefore, N_1 and N_2 share the same leaves on sides T and W (modulo swapping the two sides). Since the two networks also induce the same circular ordering of $\mathcal{X}$, they also share the same leaves on sides $X \cup Y$ and $U \cup V$ (modulo swapping these two sets of sides). We now consider two subcases.

Case 3.1: $|\Sigma| \leq 1$ for all $\Sigma \in \{U, V, X, Y\}$ for N_1 and N_2. In this case, we have that $|\mathcal{X}| \leq 6$, while we already showed that we may assume that $|\mathcal{X}| \geq 5$. When $|\mathcal{X}| = 6$, N_1 and N_2 cannot be distinct while sharing the same leaves below the reticulation nodes, sharing the same leaves on sides $U \cup V$ and $X \cup Y$ (modulo swapping these two sets of sides), and sharing the same circular ordering. Hence, we get that $|\mathcal{X}| = 5$. Then, the networks N_1 and N_2 are the two distinct quinnets from Figure 6 (up to relabeling the leaves). Thus, N_1 and N_2 are distinguishable by Lemma 2.14d.

Case 3.2: $|\Sigma| \geq 2$ for some $\Sigma \in \{U, V, X, Y\}$ for N_1 or N_2. Without loss of generality, assume that $|U| \geq 2$ for N_1. Now let t be the leaf on side T of N_1 and set $S = \mathcal{X} \setminus \{t\}$. Then, the tree-of-blobs of the network $N_1|_S$ will induce a nontrivial split that cuts off the leaves on side U of N_1 from the rest. If the tree-of-blobs of $N_2|_S$ is not isomorphic, the two networks are distinguishable by Corollary 2.12. Thus, we can assume they are isomorphic and the tree-of-blobs of $N_2|_S$ also induces the same split. But since N_1 and N_2 already share the same leaves on sides $U \cup V$, this means that they share the same leaves on side U and on side V. If $|X| \geq 2$ or $|Y| \geq 2$ for N_1 or N_2, we can repeat this argument to obtain distinguishability between the two networks. Hence, we may assume that $|X| \leq 1$ and $|Y| \leq 1$ for both networks. Recall that the two networks already share the same leaves on sides $X \cup Y$ and that they are ordered the same. If both sets have size 1, it follows that the two networks are equal: a contradiction. Thus, for the two networks to be distinct, we assume without loss of generality that $|X| = 1$ and $|Y| = 0$ for N_1, while $|X| = 0$ and $|Y| = 1$ for N_2. Now, let σ be the leaf that is on the sides $X \cup Y$ of the two networks. Furthermore, let t be the leaf on side T of the networks, w the leaf on side W of the networks, u a leaf on side U of the networks, and v a leaf on side V of the networks (which both exist, since N_1 is triangle-free and its side Y is empty, while N_2 is triangle-free and its side X is empty). Then, $N_1|_S$ and $N_2|_S$ with $S = \{\sigma, t, u, v, w\}$ are the two distinct quinnets from Figure 6 (up to relabeling the leaves). Thus, N_1 and N_2 are distinguishable by Lemma 2.14d. $\square$