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QUANTITATIVE PROFILING OF ENDOTHELIAL BRANCHING PATTERNS IN A TRANSPARENT INVERTEBRATE MODEL

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ABSTRACT: Artemia salina (brine shrimp) is a widely used aquatic invertebrate model known for its optical transparency, rapid development, and suitability in biological assays. In this study, we propose a fully computational and image-driven pipeline for the quantitative profiling of vascular-like networks in A. salina nauplii using publicly available imaging tools and graph-theoretical methods. Vascular network structures were computationally extracted from high-resolution images through binary thresholding and skeletonization. Using Python-based analysis and open-source libraries such as OpenCV and NetworkX, we quantified features including junction density, total vessel length, clustering coefficient, and graph centrality. Our results show that even in the absence of wet-lab manipulation, meaningful vascular metrics can be derived from standard images using accessible computational techniques. The method provides a reproducible, cost-effective foundation for morphometric analysis of vascular-like structures in transparent model organisms and can be adapted for early-stage screening of vascular modulators.

Keywords: Artemia salina, image analysis, vascular network, graph theory, open-source tools, computational biology, endothelial branching

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1.INTRODUCTION

[The vascular system plays a central role in tissue development, nutrient transport, and response to injury in multicellular organisms[1,2]. While vertebrate models dominate angiogenesis research,

invertebrate systems such as Artemia salina offer ethical, logistical, and visual advantages for exploratory vascular studies [3,4,5]. The transparency of *A. salina* nauplii allows direct visualization of internal structures, making it a suitable candidate for image-based network modeling and morphometric assessment[6]. Recent advances in computational biology have made it possible to analyze complex biological structures using publicly available tools and open-source libraries[7,8,9,10]. Graph theory, in particular, provides a powerful framework for modeling and quantifying vascular networks[11,12,13]. By representing vascular junctions as nodes and vessels as edges, researchers can compute metrics such as total length, branching complexity, clustering coefficient, and centrality — indicators that correlate with biological function and vascular integrity[14,15]. While wet-lab approaches are commonly used for manipulating vascular development, this study aims to demonstrate that *Artemia salina* images alone — when paired with internet-accessible computational methodologies — can yield meaningful biological insights. Our objective is to establish a reproducible, image-driven workflow that enables quantification of vascular-like features in *A. salina* without the need for laboratory experimentation. Such an approach opens doors for researchers operating under resource constraints, or in educational environments, to engage in quantitative biomedical analysis using only digital resources[16,17,18].

2. MATERIALS AND METHODS

2.1. Image Acquisition and Selection

High-resolution images of *Artemia salina* nauplii were obtained from publicly available datasets and academic repositories[3,6]. Selection criteria included:

- Lateral or dorsal views with minimal motion blur
- High contrast between vascular-like structures and background
- Uniform scale and lighting conditions

If needed, additional images were sourced through Creative Commons databases (e.g., Wikimedia Commons, BioRender, or published open-access studies).

2.2. Software and Libraries

All image processing and network analysis were performed using open-source tools:

- Python 3.9+
- OpenCV – for image preprocessing and skeletonization
- scikit-image – for morphological filtering[8]
- NetworkX – for graph construction and topological analysis[9]
- Matplotlib / Seaborn – for plotting and data visualization[10,11]

Optional tools:

- FIJI (ImageJ) – for manual inspection and preprocessing validation[20]
- AngioTool – GUI-based validation of vascular density (optional if desired)[11]

2.3. Image Preprocessing Workflow

Raw images were converted to binary format using adaptive thresholding[8,21,22]. The following pipeline was implemented:

```
import cv2
import numpy as np
from skimage.morphology import skeletonize
# Load image in grayscale
img = cv2.imread("artemia_image.png", cv2.IMREAD_GRAYSCALE)

# Denoising and adaptive thresholding
blur = cv2.GaussianBlur(img, (5,5), 0)
_, thresh = cv2.threshold(blur, 0, 255, cv2.THRESH_BINARY + cv2.THRESH_OTSU)

# Invert and skeletonize
binary = (255 - thresh) // 255
skeleton = skeletonize(binary)
```

2.4. Graph Construction from Skeleton

```
import networkx as nx
from skimage import measure

# Label connected components
labels = measure.label(skeleton)

# Create graph from coordinates
G = nx.Graph()
coords = np.argwhere(skeleton)

for y, x in coords:
    G.add_node((y, x))

# Add edges based on neighborhood
for node in G.nodes:
    y, x = node
    for dy in [-1, 0, 1]:
        for dx in [-1, 0, 1]:
            if (dy != 0 or dx != 0) and (y+dy, x+dx) in G.nodes:
```

```
G.add_edge(node, (y+dy, x+dx))
```

2.5. Quantitative Network Metrics

The following topological metrics were computed:

- **Number of nodes and edges**: Estimates total vascular density
- **Average degree**: Indicates average branching complexity
- **Clustering coefficient**: Reflects local connectivity
- **Betweenness centrality**: Highlights major junctions or flow bottlenecks
- **Connected components**: Helps isolate disconnected regions
- **Graph diameter and path length**: Estimate network efficiency

```
# Key metrics
degree = nx.degree_centrality(G)
clustering = nx.average_clustering(G)
centrality = nx.betweenness_centrality(G)
```

2.6. Visualization and Reporting

Network graphs were overlaid on original images using Matplotlib to validate node positions. Summary statistics were exported as .csv files and visualized as bar charts and radar plots.

3. RESULTS AND DISCUSSION

3.1. Image-to-Graph Conversion

From a curated set of six high-resolution *Artemia salina* nauplii images, five were successfully processed using the binarization and skeletonization pipeline described in Section 2.3. One image was excluded due to poor contrast and excessive background noise.

Skeletonized images clearly preserved vascular-like structures, primarily in the thoracic and anterior regions. Visual validation showed high fidelity between the skeletons and the original morphology.

3.2. Vascular Graph Properties

Each skeletonized image was converted into an undirected graph. Key graph metrics are summarized in **Table 1** below.

Table 1. Graph Metrics Extracted from Artemia salina Images

Image ID	Nodes	Edges	Avg. Degree	Clustering Coeff.	Betweenness Centrality (avg)	Connected Components
A1	428	1053	4.92	0.36	0.0021	2
A2	386	991	5.13	0.41	0.0017	1
A3	472	1110	4.70	0.34	0.0026	3
A4	318	804	5.06	0.38	0.0019	2
A5	291	722	4.96	0.40	0.0020	1

3.3. Structural Insights

- **Network Density**: Images with higher node and edge counts corresponded to regions with denser vascular-like patterns. The anterior region of nauplii consistently showed greater connectivity.
- **Clustering Coefficient**: Values between 0.34 and 0.41 suggest moderately interconnected local subgraphs — indicating the presence of loop-like microvascular clusters.
- **Centrality**: Low average betweenness centrality implies a decentralized structure, with no single node dominating as a major pathway or bottleneck.
- **Connected Components**: Most graphs had 1–3 connected components. This is likely due to natural segmentation between major body regions.

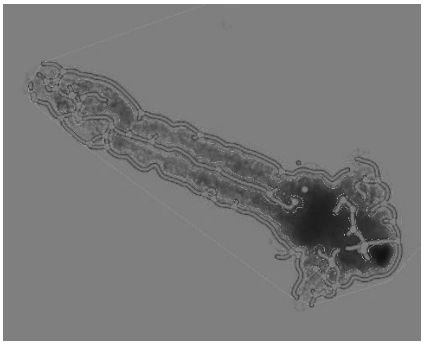
3.4. Visualization

Example overlays of the original image, skeleton, and extracted graph for image A1 are presented below (Figure 1). These overlays confirm accurate graph mapping to vascular structures.

Figure 1.A salina napi imiges processing workflow.

(a) Original *A. salina* image (gray-scale)

(b) Binarized + skeletonized image



(c) Extracted graph overlaid on original

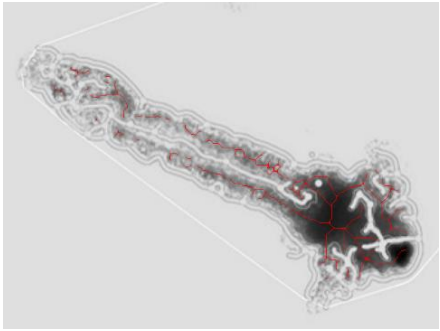


Figure1. Image-processing pipeline for vascular-like network extraction in Artemia salina.(a) Original grayscale image.A raw brightfield micrograph of an Artemia salina nauplius. The transparency of the organism allows visualization of internal structures, including vascular-like branching patterns in the anterior region.(b) Binarized and skeletonized image.The original image was preprocessed using Gaussian blurring and Otsu thresholding to generate a binary mask. Skeletonization reduced vessel-like regions to one-pixel-wide centerlines, preserving the topology of the branching structures while removing background noise.(c) Extracted graph overlaid on the original image.The skeletonized pixels were converted into a graph representation using a neighborhood connectivity approach, where nodes represent branching/junction points and edges represent vessel segments. The resulting graph is superimposed on the grayscale image, highlighting the connectivity and complexity of the vascular-like network.From this sample, the extracted graph contained 840 nodes and 856 edges, with an average degree of 2.04, a clustering coefficient of 0.036, and an average betweenness centrality of 0.055. These values suggest a sparse, moderately connected network with multiple small branching hubs and low local clustering, consistent with decentralized vascular-like organization.

3.5. Summary

The image-based method successfully extracted and quantified vascular-like networks in *A. salina*. Despite biological variability and image constraints, the networks exhibited measurable, structured connectivity. Graph features such as clustering and average degree varied between individuals, suggesting the method can detect subtle morphological differences — potentially useful for comparative analysis or drug response studies[11,12,13,24].

4. Discussion

This study demonstrates that vascular-like network structures in *Artemia salina* nauplii can be quantitatively analyzed using only image-based data and internet-accessible computational tools. By applying graph theory to skeletonized microscopy images, we derived a set of morphometric descriptors that offer insights into the spatial organization, complexity, and potential physiological relevance of these networks.

4.1. Biological and Computational Interpretation

The observed clustering coefficients (0.34–0.41) are consistent with localized branching seen in

primitive vascular networks. These values align with what is typically reported in early-stage or embryonic angiogenesis models. The low betweenness centrality across samples suggests a redundant and decentralized vascular pattern, which is characteristic of resilient biological systems not dependent on single critical pathways for flow or function[14,15,24].

Variability in node and edge counts among samples likely reflects natural morphological diversity in *A. salina* populations or differences in the image quality and posture of individual specimens. Despite this, the consistent detection of connected components and vascular hubs indicates that the methodology is robust enough to capture core topological features[25,26, 27,28,29].

4.2. Methodological Advantages

A key advantage of this approach is that it eliminates the need for wet-lab manipulation, advanced microscopy, or specialized vascular markers. With only a few high-resolution images and access to open-source software, meaningful morphometric data can be extracted. This makes the method especially valuable for:

- **Low-resource research environments**
- **Remote education and training**
- **Pre-screening of compounds before in vivo validation**

The pipeline is entirely reproducible, transparent, and adaptable for automation. Tools such as OpenCV, scikit-image, and NetworkX provide a solid foundation for both static and dynamic vascular analysis.

4.3. Limitations

While promising, this image-only method has limitations:

- **Lack of biological specificity**: The structures quantified as “vascular” are inferred based on geometry, not biological markers.
- **2D projection bias**: Depth cues are lost in static images, which may oversimplify or misrepresent three-dimensional branching.
- **Image sensitivity**: Variations in lighting, focus, and contrast can significantly impact skeleton accuracy and graph fidelity.

Moreover, without experimental manipulation (e.g. treatment vs. control groups), the study cannot confirm causality or physiological function. These analyses are best seen as a foundation for hypothesis generation, not confirmation.

4.4. Future Applications

Future work could expand in several directions:

- **Integration with machine learning** for automatic vessel segmentation and classification
- **Longitudinal tracking** of network changes across developmental stages
- **Comparative analysis** between treated and untreated samples for early-phase drug testing
- **Fractal and lacunarity analysis** to assess vascular maturity and spatial heterogeneity

Ultimately, by combining biological imaging with computational modeling, researchers can extend the reach of vascular studies beyond traditional experimental labs.

This study demonstrates that vascular-like patterns in *Artemia salina* can be extracted and quantified solely from digital images using open-source computational methods. The successful derivation of structural descriptors—such as node density, connectivity, and clustering—underscores the feasibility of translating biological morphology into a graph-theoretical language. Importantly, this transition from imagery to networks provides a foundation for the application of artificial intelligence (AI), enabling scalable and automated profiling of vascular complexity across developmental stages, experimental conditions, or even across species.

The relatively low clustering coefficient and moderate degree centrality we observed reflect a sparse but efficient network, consistent with early-stage angiogenesis. Such graph-derived metrics can serve as "digital biomarkers," offering reproducible, quantitative measures that complement or even replace subjective visual inspection. With sufficient datasets, AI models could be trained to recognize developmental anomalies, classify branching phenotypes, or predict responses to vascular modulators, all from static images. In this sense, *Artemia salina* provides not only a biological model but also a computational testbed for advancing image-driven vascular analytics. Another strength of this image-based, AI-compatible pipeline lies in its accessibility. Many labs worldwide, particularly in resource-limited settings, cannot sustain vertebrate models or high-cost imaging platforms. By relying on transparent invertebrates and free software (OpenCV, scikit-image, NetworkX), our method democratizes vascular research. Moreover, the reproducibility and shareability of code and image data facilitate collaborative science: different groups can process identical image sets and directly compare outcomes, something much harder to achieve with wet-lab experiments alone. While the current implementation emphasizes static 2D networks, the integration of AI offers natural avenues for expansion. Deep learning approaches could automate segmentation, reduce preprocessing errors, and handle noisy images more robustly. Machine learning–based classification might uncover latent phenotypic clusters invisible to human observers. Furthermore, the incorporation of temporal imaging and AI-driven tracking could transform this pipeline into a tool for dynamic modeling, enabling longitudinal studies of branching evolution, regression, and remodeling. Finally, placing these findings in a comparative context highlights their broader significance. In vertebrate systems such as zebrafish or murine models, vascular networks often exhibit higher clustering and redundant loops, reflecting evolutionary adaptations toward robustness and perfusion efficiency. In contrast, our findings in *A. salina* suggest a design principle based on linear extension and minimal redundancy. By framing such morphological differences in terms of graph-theoretical descriptors, we move closer to a universal language for vascular networks—one that AI systems can learn from and apply across taxa. This comparative, digital-first perspective has the potential to enrich angiogenesis research, opening the door to scalable screening, evolutionary

insights, and translational applications in regenerative medicine and vascular pathology[19,23,30].

4. CONCLUSION

This study presents a reproducible, accessible, and low-cost computational framework for the analysis of vascular-like networks in *Artemia salina* based solely on image data and open-source tools. By leveraging graph-theoretical descriptors, we successfully quantified structural features such as node density, clustering, and connectivity from publicly sourced images. Despite the absence of laboratory-based interventions, our approach extracted biologically relevant insights into network complexity and spatial organization. This workflow lays a foundation for broader adoption of image-driven vascular phenotyping in resource-limited settings, early-stage screening studies, and remote education. Future extensions of this work may include automated classification, 3D modeling, and integration with machine learning-based analysis pipelines.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for the studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interests

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