Perform Network Analysis

- Create a covariance matrix
- Find an ideal noise-removing threshold filter with Random Matrix Theory
- Analyze properties of the network

TODO: Split Dataset into Sample Groups

Network Construction

- create a pair-wise similarity (Pearson) of abundance across different samples
- determine adjacency matrix by RMT-based approach

In [8]:
```
import pandas as pd
import numpy as np
import scipy
import matplotlib.pyplot as plt
```

In [9]:
```
from numpy import linalg as LA
```

In [10]:
```
import empyricalRMT as rmt
from empyricalRMT.construct import generate_eigs
from empyricalRMT.eigenvalues import Eigenvalues
```

Import Data

In [11]:
```
# load data
df_counts_rel = pd.read_csv("data/FCF_relative_counts.csv", index_col=0)
df_annotations = pd.read_csv("data/FCF_annotations_corrected.csv", index_col=0)

print("df_counts_rel.shape", df_counts_rel.shape)
print("df_annotations.shape", df_annotations.shape)
```
```
df_counts_rel.shape (87, 60)
df_annotations.shape (87, 4)
```

Create A Covariance Matrix

In [12]:
```
# make pearson correlation matrix
df_counts_rel_corr = df_counts_rel.corr(method="pearson")

# make similarity data
S_corr = df_counts_rel_corr.abs()

# set an initial threshold value
s_tb = 0.05
```

Use Random Matrix Theory

Good overview of Random Matrix Theory by Torsten Scholak

TODO: figure out if my Poisson distribution is very off... my chi_squared value seems unreal...

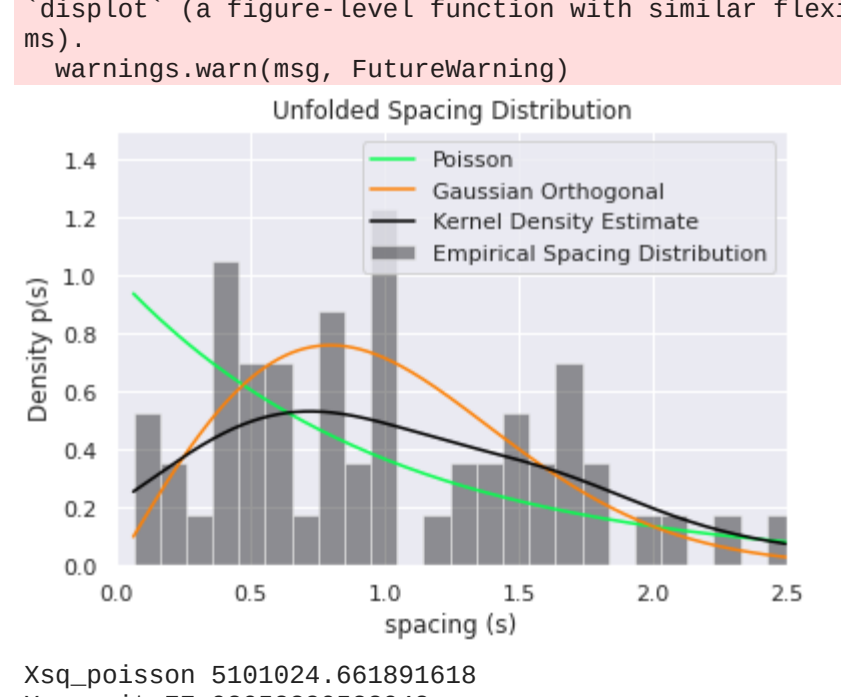
In [13]:
```
def get_thresholded_matrix(S_corr, s_t, verbose=False):
    # apply threshold
    A = S_corr >= s_t
    A = A.astype(int)
    # replace values on diagonals with 0
    np.fill_diagonal(A.values, 0)
    # keep non-zero rows and columns
    A = A.loc[:, (A != 0).any(axis=0)]
    A = A.loc[(A != 0).any(axis=1), :]
    if verbose:
        # get a sense of how many OTUs remain
        print("A.shape", A.shape)
    return A


def get_unfolded_eigs(S_corr, s_t, verbose=False):
    A = get_thresholded_matrix(S_corr, s_t)

    # calculate eigenvalues
    w, _ = LA.eig(A)
    # sort eigenvalues
    # To test NNSD distribution, order the eigenvalue, smallest first
    w_sorted = np.sort(w)

    # use empiricalRMT library
    eigs = Eigenvalues(w_sorted)

    # unfold the eigenvalues by fitting to cubic spline
    unfolded = eigs.unfold(smoother="poly", degree=3)

    if verbose:
        # plot some classic observables and compare to theory
        ensembles = ["poisson", "goe"]  # theoretically expected curves to plot
        unfolded.plot_nnsd(ensembles=ensembles)  # nearest neighbours spacings

    return unfolded.vals


def get_expected_poisson(unfolded):
    p = np.pi
    _spacings = np.diff(unfolded)
    s = np.linspace(_spacings.min(), _spacings.max(), len(unfolded))
    poisson = np.exp(-s)
    return poisson


def get_expected_GOE(unfolded):
    p = np.pi
    _spacings = np.diff(unfolded)
    s = np.linspace(_spacings.min(), _spacings.max(), len(unfolded))
    goe = ((p * s) / 2) * np.exp(-(p / 4) * s * s)
    return goe


def chi_squared(observed, expected):
    d = (observed-expected)**2
    d = d/expected
    d = d.sum()
    return d


def find_threshold(S_corr, s_tb, alpha=0.05):
    """
    Use Random Matrix Theory to find a threshold to
    separate random noise (following GOE NNSD)
    from non-random signal (following Poisson NNSD).

    H0: P(d) follows the Poisson distribution
    H1: P(d) does not follow the Poisson distribution
    """
    # set significance threshold
    s_t = s_tb

    coarse_step = 0.1
    fine_step = 0.01
    is_finding_finer_theshold = False

    s_t_vals = []
    unfolded_len_vals = []
    Xsq_crit_vals = []
    Xsq_poisson_vals = []
    Xsq_GOE_vals = []

    while(s_t < 1.0):
        unfolded = get_unfolded_eigs(S_corr, s_t, verbose=False)
        Xsq_crit = scipy.stats.chi2.ppf(1-alpha, df=len(unfolded)-1)

        # chi-square for poisson distribution
        expected_poisson = get_expected_poisson(unfolded)
        Xsq_poisson = chi_squared(unfolded, expected_poisson)

        # chi-square for gaussian distribution
        expected_GOE = get_expected_GOE(unfolded)
        Xsq_GOE = chi_squared(unfolded, expected_GOE)

        # savepoint
        s_t_vals.append(s_t)
        unfolded_len_vals.append(len(unfolded))
        Xsq_crit_vals.append(Xsq_crit)
        Xsq_poisson_vals.append(Xsq_poisson)
        Xsq_GOE_vals.append(Xsq_GOE)

        # savepoint
        print("s_t", s_t)
        print("len(unfolded)", len(unfolded))
        print("Xsq_crit", Xsq_crit)
        print("Xsq_poisson", Xsq_poisson)
        print("Xsq_GOE", Xsq_GOE)

        if (is_finding_finer_theshold):
            if (Xsq_poisson > Xsq_crit):
                print("--accept Poisson")
                # reject null hypothesis that NNSD is Poisson
                s_t += fine_step

            else:
                # do not reject null hypothesis that NNSD is Poisson
                break

        else:
            if (Xsq_poisson > Xsq_crit):
                # reject null hypothesis that NNSD is Poisson
                print("--reject Poisson")
                s_t += coarse_step

            else:
                # do not reject null hypothesis that NNSD is Poisson
                # find a finer threshold
                s_t -= coarse_step
                is_finding_finer_theshold = True

    print("s_t_vals", s_t_vals)
    print("unfolded_len_vals", unfolded_len_vals)
    print("Xsq_crit_vals", Xsq_crit_vals)
    print("Xsq_poisson_vals", Xsq_poisson_vals)
    print("Xsq_GOE_vals", Xsq_GOE_vals)

    return s_t
```

In [14]:
```
find_threshold(S_corr, 0.3, alpha=0.5)
```
```
s_t 0.3
len(unfolded) 60
Xsq_crit 58.33468914643707
Xsq_poisson 7770300.954883633
Xsq_GOE inf
--reject Poisson
s_t 0.4
len(unfolded) 60
Xsq_crit 58.33468914643707
Xsq_poisson 5188369.444913099
Xsq_GOE inf
--reject Poisson
s_t 0.5
len(unfolded) 59
Xsq_crit 57.334712814818765
Xsq_poisson 7113043.838085005
Xsq_GOE inf
--reject Poisson
s_t 0.6
len(unfolded) 53
Xsq_crit 51.334874205924834
Xsq_poisson (11472979.837415377-8.696346172319038e-11j)
Xsq_GOE (inf-infj)
--reject Poisson
s_t 0.7
len(unfolded) 41
Xsq_crit 39.33534484661134
Xsq_poisson 2925742.812956208
Xsq_GOE inf
--reject Poisson
s_t 0.7999999999999999
len(unfolded) 32
Xsq_crit 30.33594245819813
Xsq_poisson 1584963.4244634905
Xsq_GOE inf
--reject Poisson
s_t 0.8999999999999999
len(unfolded) 15
Xsq_crit 13.33927414909954
Xsq_poisson 194079.37872329363
Xsq_GOE inf
--reject Poisson
```
```
<ipython-input-13-a853e8cc8e28>:57: RuntimeWarning: divide by zero encountered in true_divide
  d = d/expected
<ipython-input-13-a853e8cc8e28>:57: RuntimeWarning: divide by zero encountered in true_divide
  d = d/expected
<ipython-input-13-a853e8cc8e28>:57: RuntimeWarning: divide by zero encountered in true_divide
  d = d/expected
/home/gaher/miniconda3/envs/MBEnv/lib/python3.8/site-packages/empyricalRMT/_validate.py:9: ComplexWarning: Cast
ing complex values to real discards the imaginary part
  arr = np.array(array, dtype=float)
<ipython-input-13-a853e8cc8e28>:57: RuntimeWarning: divide by zero encountered in true_divide
  d = d/expected
<ipython-input-13-a853e8cc8e28>:57: RuntimeWarning: divide by zero encountered in true_divide
  d = d/expected
<ipython-input-13-a853e8cc8e28>:57: RuntimeWarning: divide by zero encountered in true_divide
  d = d/expected
/home/gaher/miniconda3/envs/MBEnv/lib/python3.8/site-packages/numpy/polynomial/polynomial.py:1336: RankWarning:
The fit may be poorly conditioned
  return pu._fit(polyvander, x, y, deg, rcond, full, w)
<ipython-input-13-a853e8cc8e28>:57: RuntimeWarning: divide by zero encountered in true_divide
  d = d/expected
---------------------------------------------------------------------------
TypeError                                 Traceback (most recent call last)
<ipython-input-14-e3555a59c00b> in <module>
----> 1 find_threshold(S_corr, 0.3, alpha=0.5)

<ipython-input-13-a853e8cc8e28> in find_threshold(S_corr, s_tb, alpha)
     84
     85     while(s_t < 1.0):
---> 86         unfolded = get_unfolded_eigs(S_corr, s_t, verbose=False)
     87         Xsq_crit = scipy.stats.chi2.ppf(1-alpha, df=len(unfolded)-1)
     88

<ipython-input-13-a853e8cc8e28> in get_unfolded_eigs(S_corr, s_t, verbose)
     27
     28     # unfold the eigenvalues by fitting to cubic spline
---> 29     unfolded = eigs.unfold(smoother="poly", degree=3)
     30
     31     if verbose:

~/miniconda3/envs/MBEnv/lib/python3.8/site-packages/empyricalRMT/eigenvalues.py in unfold(self, smoother, degre
e, spline_smooth, detrend)
    689
    690         eigs = self.eigs
--> 691         unfolded, _, closure = Smoother(eigs).fit(
    692             smoother=smoother,
    693             degree=degree,

~/miniconda3/envs/MBEnv/lib/python3.8/site-packages/empyricalRMT/smoother.py in fit(self, smoother, degree, spl
ine_smooth, detrend, return_callable)
     98
     99         if smoother == "poly":
--> 100             poly_coef = polyfit(eigs, steps, degree)
    101             unfolded = polyval(eigs, poly_coef)
    102             func = lambda x: polyval(x, poly_coef) if return_callable else None

~/miniconda3/envs/MBEnv/lib/python3.8/site-packages/numpy/polynomial/polynomial.py in polyfit(x, y, deg, rcond,
 full, w)
   1334
   1335     """
-> 1336     return pu._fit(polyvander, x, y, deg, rcond, full, w)
   1337
   1338

~/miniconda3/envs/MBEnv/lib/python3.8/site-packages/numpy/polynomial/polyutils.py in _fit(vander_f, x, y, deg,
 rcond, full, w)
    666         raise TypeError("expected 1D vector for x")
    667     if x.size == 0:
--> 668         raise TypeError("expected non-empty vector for x")
    669     if y.ndim < 1 or y.ndim > 2:
    670         raise TypeError("expected 1D or 2D array for y")

TypeError: expected non-empty vector for x
```

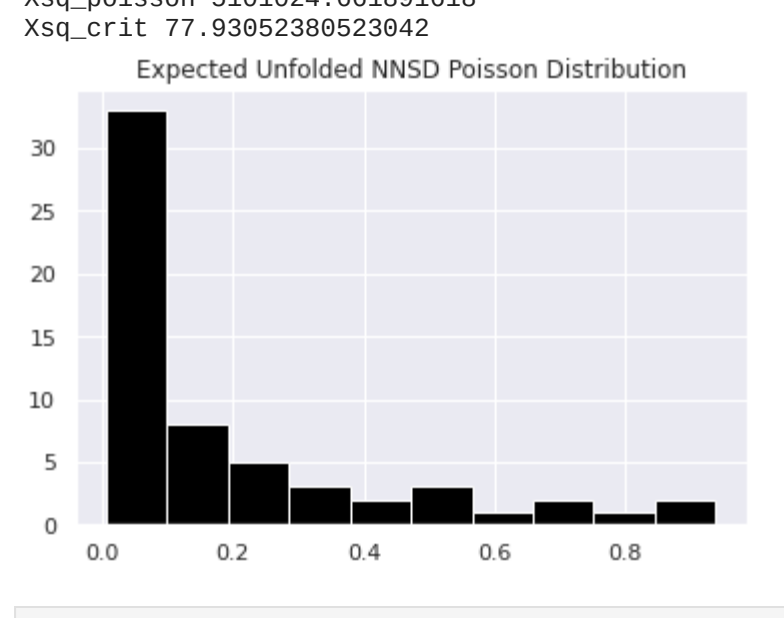
Data Is Too Messy

Since the data is too messy, this code lets you eyeball the threshold where the data fits the Poisson distribution more than it fits the GOE distribution.

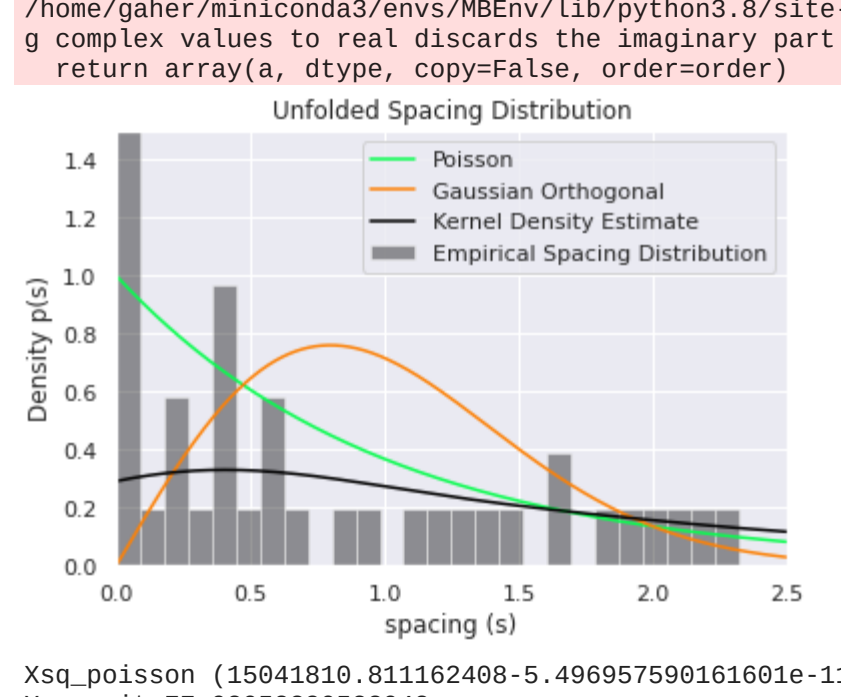
In [36]:
```
visualize_threshold(S_corr, 0.03)
```
```
/home/gaher/miniconda3/envs/MBEnv/lib/python3.8/site-packages/seaborn/distributions.py:2551: FutureWarning: `di
stplot` is a deprecated function and will be removed in a future version. Please adapt your code to use either
`displot` (a figure-level function with similar flexibility) or `histplot` (an axes-level function for histogra
ms).
  warnings.warn(msg, FutureWarning)
```



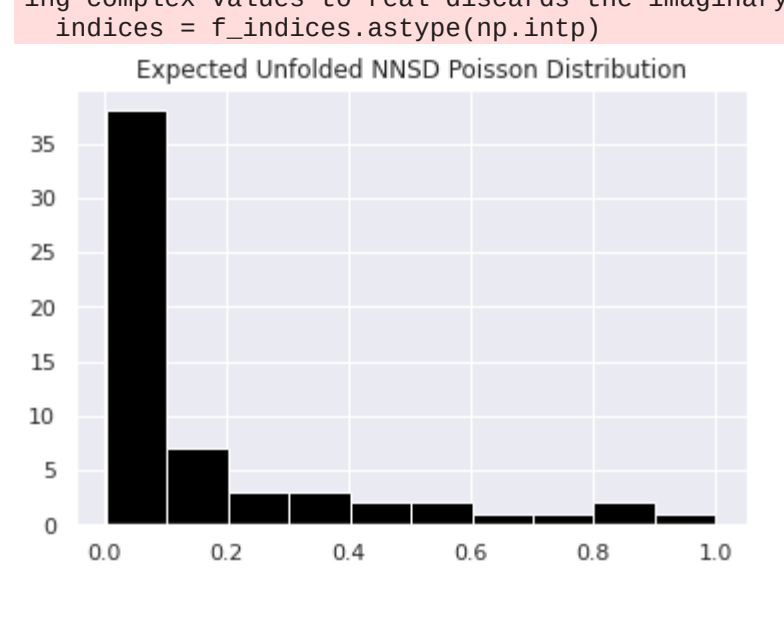

```
Xsq_poisson 5101024.661891618
Xsq_crit 77.93052380523042
```



In [15]:
```
def visualize_threshold(S_corr, theshold):
    unfolded = get_unfolded_eigs(S_corr, theshold, verbose=True)

    Xsq_crit = scipy.stats.chi2.ppf(1-0.05, df=len(unfolded)-1)

    # chi-square for poisson distribution
    expected_poisson = get_expected_poisson(unfolded)
    Xsq_poisson = chi_squared(unfolded, expected_poisson)

    print("Xsq_poisson", Xsq_poisson)
    print("Xsq_crit", Xsq_crit)

    plt.figure()
    plt.hist(get_expected_poisson(unfolded))
    plt.title("Expected Unfolded NNSD Poisson Distribution")

visualize_threshold(S_corr, 0.47)
```
```
/home/gaher/miniconda3/envs/MBEnv/lib/python3.8/site-packages/empyricalRMT/_validate.py:9: ComplexWarning: Cast
ing complex values to real discards the imaginary part
  arr = np.array(array, dtype=float)
/home/gaher/miniconda3/envs/MBEnv/lib/python3.8/site-packages/seaborn/distributions.py:2551: FutureWarning: `di
stplot` is a deprecated function and will be removed in a future version. Please adapt your code to use either
`displot` (a figure-level function with similar flexibility) or `histplot` (an axes-level function for histogra
ms).
  warnings.warn(msg, FutureWarning)
/home/gaher/miniconda3/envs/MBEnv/lib/python3.8/site-packages/numpy/core/_asarray.py:85: ComplexWarning: Castin
g complex values to real discards the imaginary part
  return array(a, dtype, copy=False, order=order)
/home/gaher/miniconda3/envs/MBEnv/lib/python3.8/site-packages/numpy/core/_asarray.py:85: ComplexWarning: Castin
g complex values to real discards the imaginary part
  return array(a, dtype, copy=False, order=order)
/home/gaher/miniconda3/envs/MBEnv/lib/python3.8/site-packages/numpy/core/_asarray.py:85: ComplexWarning: Castin
g complex values to real discards the imaginary part
  return array(a, dtype, copy=False, order=order)
/home/gaher/miniconda3/envs/MBEnv/lib/python3.8/site-packages/numpy/core/_asarray.py:85: ComplexWarning: Castin
g complex values to real discards the imaginary part
  return array(a, dtype, copy=False, order=order)
```




```
Xsq_poisson (15041810.811162408-5.496957590161601e-11j)
Xsq_crit 77.93052380523042
```
```
/home/gaher/miniconda3/envs/MBEnv/lib/python3.8/site-packages/numpy/lib/histograms.py:854: ComplexWarning: Cast
ing complex values to real discards the imaginary part
  indices = f_indices.astype(np.intp)
```



Analyze Network Properties:

Key Characteristics:

- k-core, average path length, local clustering coefficient
- the average degree, degree distribution
- module, clique, motif

Features of Nodes:

- degree, degree centrality
- node-betweenness, node-betweenness centrality
- closeness, cluster coefficient, coreness
- eigenvector centrality

Special Nodes:

- modular hub node
- connector node
- peripheral node

Features of Edges:

- shortest path, distance
- edge-betweenness

Sources:

- https://programminghistorian.org/en/lessons/exploring-and-analyzing-network-data-with-python

In [16]:
```
import networkx as nx
from networkx.algorithms import community # This part of networkx, for community detection, needs to be importe
from operator import itemgetter
```

In [17]:
```
s_t = 0.47
A = get_thresholded_matrix(S_corr, s_t)
A.shape
type(A)
```

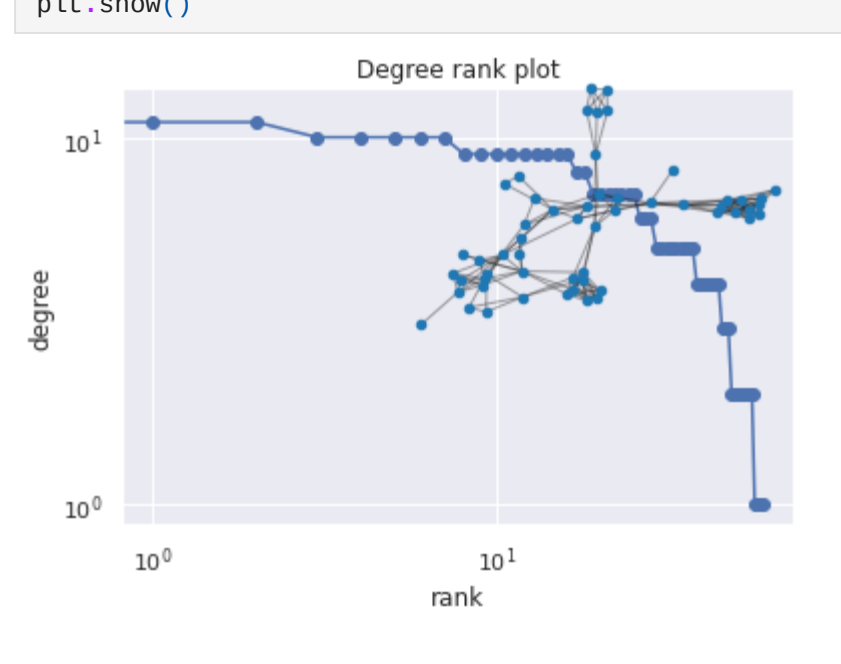
Out[17]:
```
pandas.core.frame.DataFrame
```

In [18]:
```
G = nx.from_numpy_array(A.to_numpy())

otu_dict = {}
for node in G.nodes:
    otu_dict[node] = A.index[node]
G=nx.relabel_nodes(G,otu_dict)

# nx.set_node_attributes(G, otu_dict, "OTU")
```

In [19]:
```
print(nx.info(G))
```
```
Name:
Type: Graph
Number of nodes: 60
Number of edges: 175
Average degree:   5.8333
```

Degree Plot (Useful? Probably Not)

In [20]:
```
degree_sequence = sorted([d for n, d in G.degree()], reverse=True)
dmax = max(degree_sequence)

plt.loglog(degree_sequence, "b-", marker="o")
plt.title("Degree rank plot")
plt.ylabel("degree")
plt.xlabel("rank")

# draw graph in inset
plt.axes([0.45, 0.45, 0.45, 0.45])
Gcc = G.subgraph(sorted(nx.connected_components(G), key=len, reverse=True)[0])
pos = nx.spring_layout(Gcc)
plt.axis("off")
nx.draw_networkx_nodes(Gcc, pos, node_size=20)
nx.draw_networkx_edges(Gcc, pos, alpha=0.4)
plt.show()
```



Key Characteristics

Density: tells you how connected a network is. A 0 density would mean that there are no connections at all, and a 1 would indicate that all possible edges are present (a perfectly connected network).

In [21]:
```
density = nx.density(G)
print("Network density:", density)
```
```
Network density: 0.09887005649717515
```

Transitivity: like density, expresses how interconnected a graph is in terms of a ratio of actual over possible connections. A triangle is formed if two nodes that are connected to a node are also connected to each other.

In [22]:
```
triadic_closure = nx.transitivity(G)
print("Triadic closure:", triadic_closure)
```
```
Triadic closure: 0.7005347593582888
```

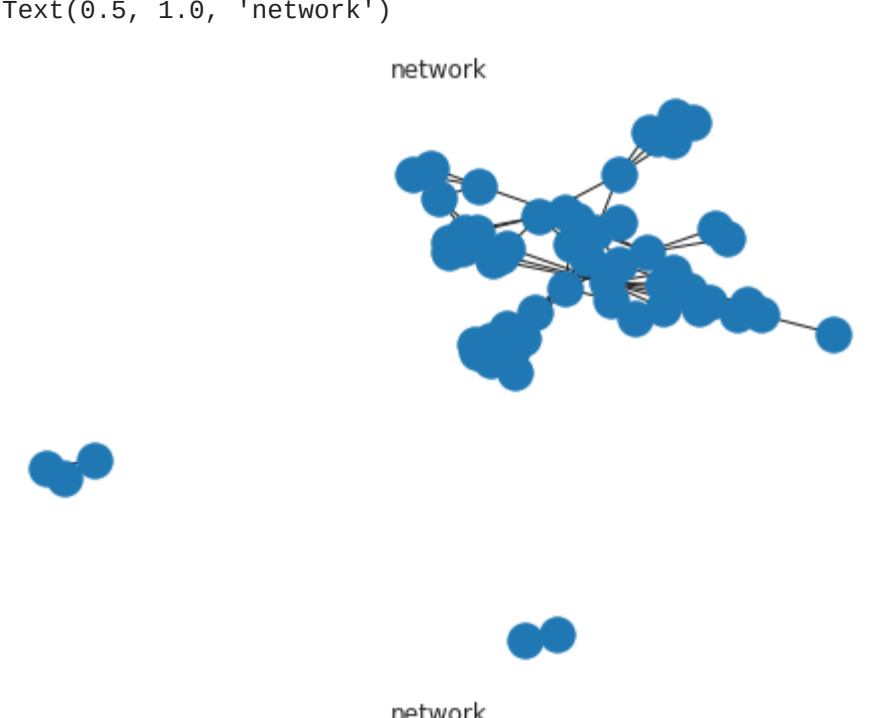
There are many network metrics derived from shortest path lengths. One such measure is **diameter**, which is the longest of all shortest paths. After calculating all shortest paths between every possible pair of nodes in the network, diameter is the length of the path between the two nodes that are furthest apart. The measure is designed to give you a sense of the network's overall size, the distance from one end of the network to another.

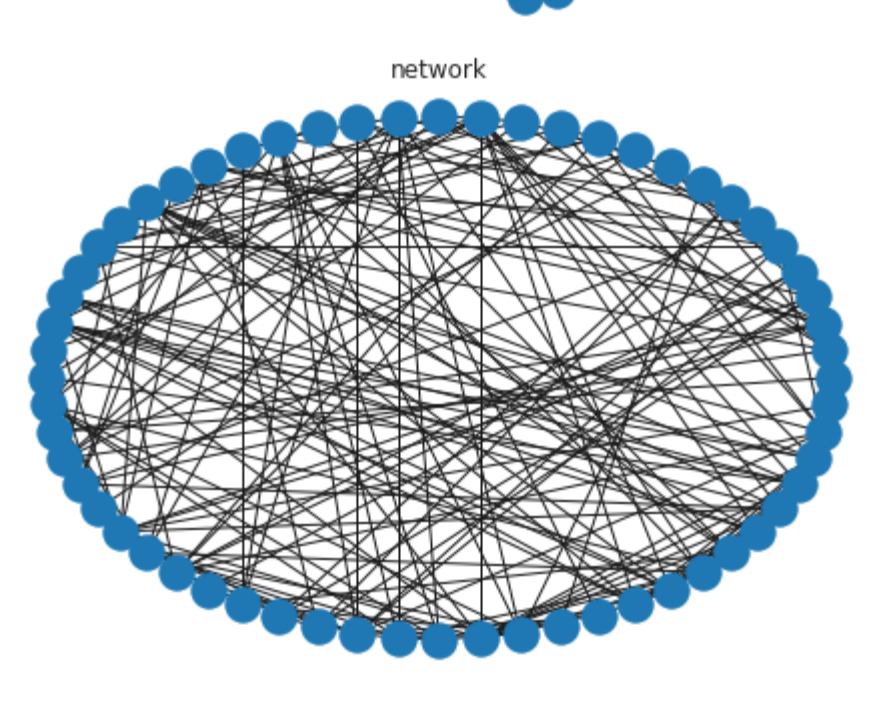
In [23]:
```
# If your Graph has more than one component, this will return False:
print(nx.is_connected(G))

# Next, use nx.connected_components to get the list of components,
# then use the max() command to find the largest one:
components = nx.connected_components(G)
largest_component = max(components, key=len)

# Create a "subgraph" of just the largest component
# Then calculate the diameter of the subgraph, just like you did with density.

subgraph = G.subgraph(largest_component)
diameter = nx.diameter(subgraph)
print("Network diameter of largest component:", diameter)
```
```
False
Network diameter of largest component: 7
```

Visualize Network

In [24]:
```
# default layout
plt.figure()
nx.draw(G)
plt.title('network')

# circular layout
plt.figure()
nx.draw_circular(G)
plt.title('network')
```

Out[24]:
```
Text(0.5, 1.0, 'network')
```





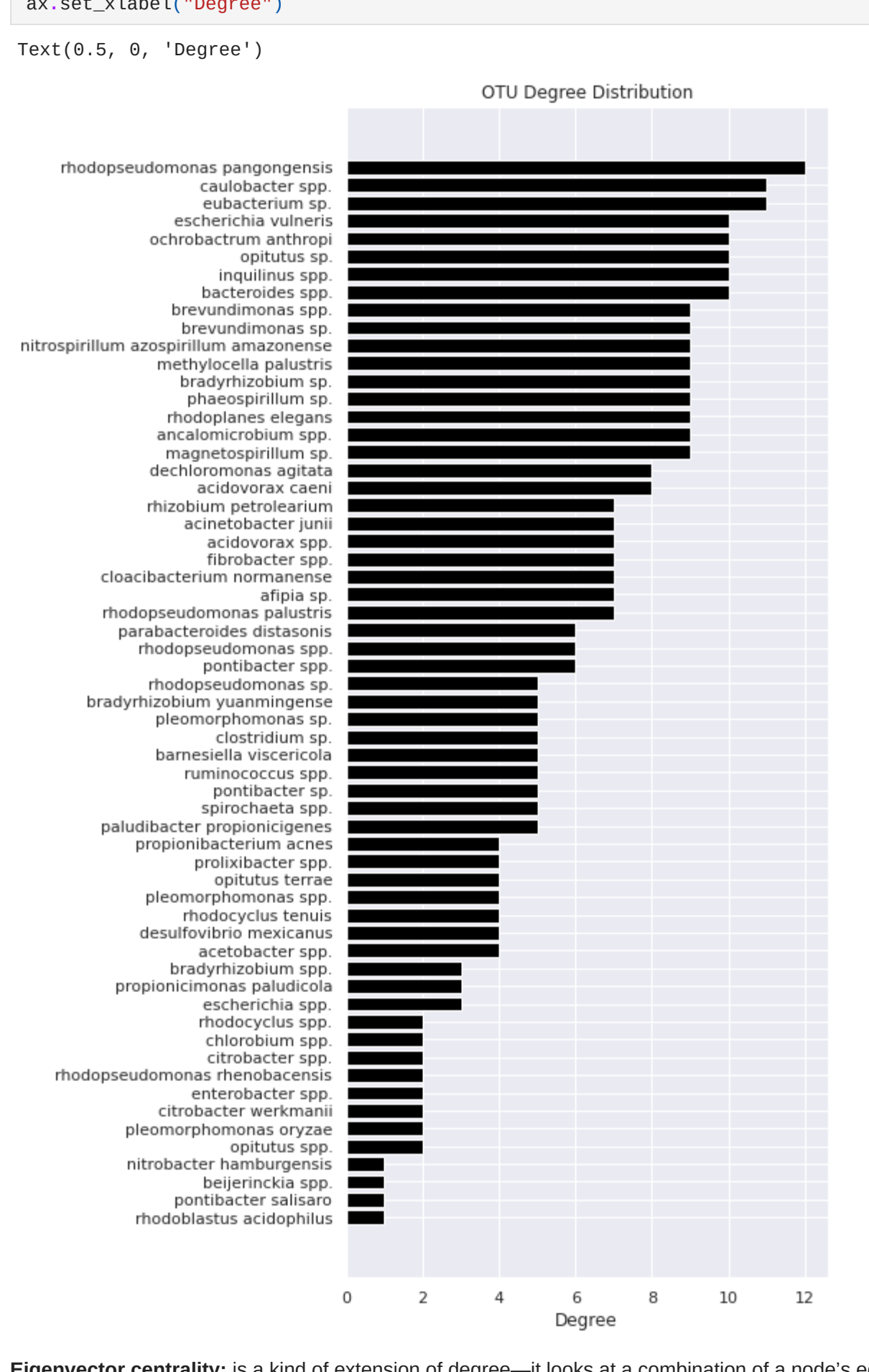
Features of Nodes

After getting some basic measures of the entire network structure, a good next step is to find which nodes are the most important ones in your network. In network analysis, measures of the importance of nodes are referred to as centrality measures. Because there are many ways of approaching the question "Which nodes are the most important?" there are many different ways of calculating centrality. Here you'll learn about three of the most common centrality measures: degree, betweenness centrality, and eigenvector centrality.

Degree Distribution: Most networks will have just a few hubs of very high degree, with the rest of similar, much lower degree

In [25]:
```
degree_dict = dict(G.degree(G.nodes()))
nx.set_node_attributes(G, degree_dict, 'degree')

sorted_degree = sorted(degree_dict.items(), key=itemgetter(1))
sorted_degree_OTU = [d[0] for d in sorted_degree]
sorted_degree_degree = [d[1] for d in sorted_degree]

f, ax = plt.subplots(figsize=(6, 15))
ax.barh(sorted_degree_OTU, width=sorted_degree_degree)
ax.set_title("OTU Degree Distribution")
ax.set_xlabel("Degree")
```

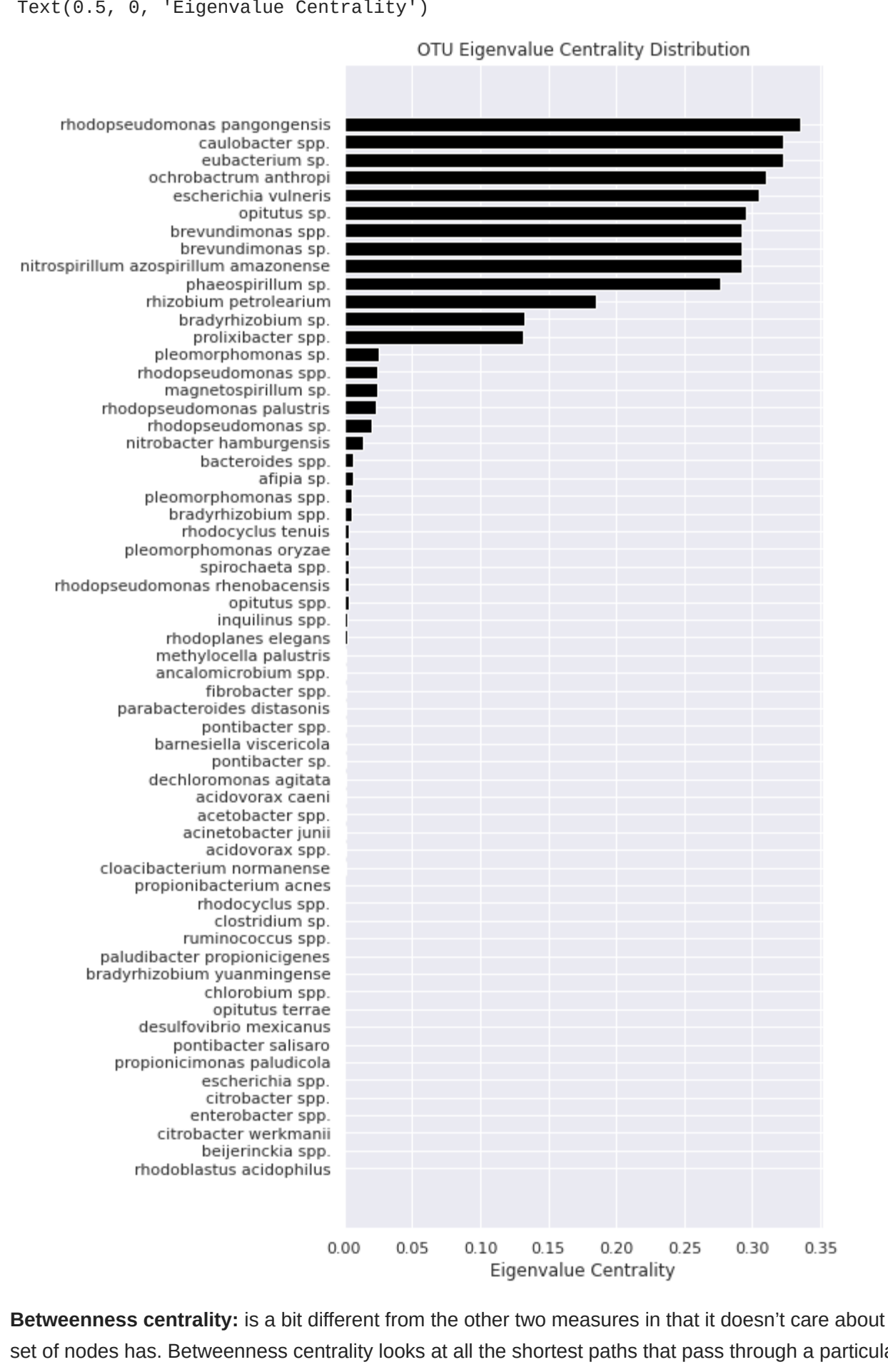
Out[25]:
```
Text(0.5, 0, 'Degree')
```



Eigenvector centrality: is a kind of extension of degree—it looks at a combination of a node's edges and the edges of that node's neighbors. Eigenvector centrality cares if you are a hub, but it also cares how many hubs you are connected to. It's calculated as a value from 0 to 1: the closer to one, the greater the centrality. Eigenvector centrality is useful for understanding which nodes can get information to many other nodes quickly. If you know a lot of well-connected people, you could spread a message very efficiently. If you've used Google, then you're already somewhat familiar with Eigenvector centrality. Their PageRank algorithm uses an extension of this formula to decide which webpages get to the top of its search results.

In [26]:
```
eigenvector_dict = nx.eigenvector_centrality(G) # Run eigenvector centrality
nx.set_node_attributes(G, eigenvector_dict, 'eigenvector')

sorted_eigcen = sorted(eigenvector_dict.items(), key=itemgetter(1))
sorted_eigcen_OTU = [d[0] for d in sorted_eigcen]
sorted_eigcen_eigcen = [d[1] for d in sorted_eigcen]

f, ax = plt.subplots(figsize=(6, 15))
ax.barh(sorted_eigcen_OTU, width=sorted_eigcen_eigcen)
ax.set_title("OTU Eigenvalue Centrality Distribution")
ax.set_xlabel("Eigenvalue Centrality")
```

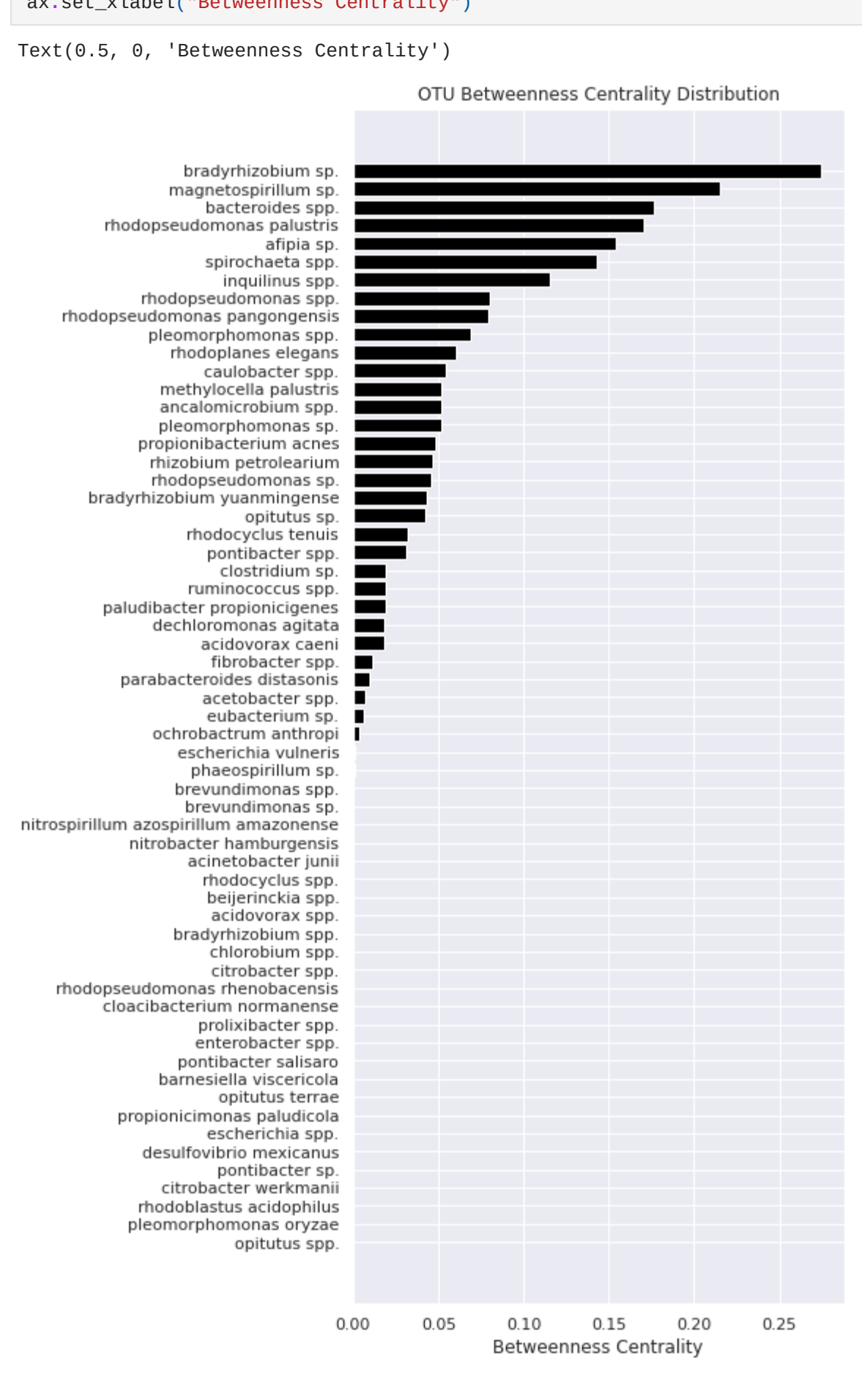
Out[26]:
```
Text(0.5, 0, 'Eigenvalue Centrality')
```



Betweenness centrality: is a bit different from the other two measures in that it doesn't care about the number of edges any one node or set of nodes has. Betweenness centrality looks at all the shortest paths that pass through a particular node (see above). To do this, it must first calculate every possible shortest path in your network, so keep in mind that betweenness centrality will take longer to calculate than other centrality measures (but it won't be an issue in a dataset of this size). Betweenness centrality, which is also expressed on a scale of 0 to 1, is fairly good at finding nodes that connect two otherwise disparate parts of a network. If you're the only thing connecting two clusters, every communication between those clusters has to pass through you. In contrast to a hub, this sort of node is often referred to as a broker. Betweenness centrality is not the only way of finding brokerage (and other methods are more systematic), but it's a quick way of giving you a sense of which nodes are important not because they have lots of connections themselves but because they stand between groups, giving the network connectivity and cohesion.

In [27]:
```
betweenness_dict = nx.betweenness_centrality(G) # Run betweenness centrality
nx.set_node_attributes(G, betweenness_dict, 'betweenness')

sorted_betcen = sorted(betweenness_dict.items(), key=itemgetter(1))
sorted_betcen_OTU = [d[0] for d in sorted_betcen]
sorted_betcen_betcen = [d[1] for d in sorted_betcen]

f, ax = plt.subplots(figsize=(6, 15))
ax.barh(sorted_betcen_OTU, width=sorted_betcen_betcen)
ax.set_title("OTU Betweenness Centrality Distribution")
ax.set_xlabel("Betweenness Centrality")
```

Out[27]:
```
Text(0.5, 0, 'Betweenness Centrality')
```



Advanced NetworkX: Community detection with modularity

There are many ways of calculating communities, cliques, and clusters in your network, but the most popular method currently is modularity. Modularity is a measure of relative density in your network: a community (called a module or modularity class) has high density relative to other nodes within its module but low density with those outside. Modularity gives you an overall score of how fractious your network is, and that score can be used to partition the network and return the individual communities.

```
In [28]: communities = community.greedy_modularity_communities(G)
         M = len(communities)
         communities
```

```
Out[28]: [frozenset({'afipia sp.',
                     'bradyrhizobium sp.',
                     'bradyrhizobium spp.',
                     'magnetospirillum sp.',
                     'nitrobacter hamburgensis',
                     'opitutus spp.',
                     'pleomorphomonas oryzae',
                     'pleomorphomonas sp.',
                     'pleomorphomonas spp.',
                     'rhodopseudomonas palustris',
                     'rhodopseudomonas rhenobacensis',
                     'rhodopseudomonas sp.',
                     'rhodopseudomonas spp.',
                     'spirochaeta spp.'}),
          frozenset({'brevundimonas sp.',
                     'brevundimonas spp.',
                     'caulobacter spp.',
                     'escherichia vulneris',
                     'eubacterium sp.',
                     'nitrospirillum azospirillum amazonense',
                     'ochrobactrum anthropi',
                     'opitutus sp.',
                     'phaeospirillum sp.',
                     'prolixibacter spp.',
                     'rhizobium petrolearium',
                     'rhodopseudomonas pangongensis'}),
          frozenset({'acetobacter spp.',
                     'bacteroides spp.',
                     'barnesiella vischericola',
                     'chlorobium spp.',
                     'fibrobacter spp.',
                     'inquilinus spp.',
                     'parabacteroides distasonis',
                     'pontibacter salisaro',
                     'pontibacter sp.',
                     'pontibacter spp.',
                     'rhodocyclus spp.',
                     'rhodocyclus tenuis'}),
          frozenset({'acidovorax caeni',
                     'acidovorax spp.',
                     'acinetobacter junii',
                     'ancalomicrobium spp.',
                     'cloacibacterium normanense',
                     'dechloromonas agitata',
                     'methylocella palustris',
                     'rhodoplanes elegans'}),
          frozenset({'clostridium sp.',
                     'desulfovibrio mexicanus',
                     'opitutus terrae',
                     'paludibacter propionicigenes',
                     'ruminococcus spp.'}),
          frozenset({'bradyrhizobium yuanmingense',
                     'escherichia spp.',
                     'propionibacterium acnes',
                     'propionicimonas paludicola'}),
          frozenset({'citrobacter spp.', 'citrobacter werkmanii', 'enterobacter spp.'}),
          frozenset({'beijerinckia spp.', 'rhodoblastus acidophilus'})]
```

```
In [29]: modularity_dict = {} # Create a blank dictionary
         for i,c in enumerate(communities): # Loop through the list of communities, keeping track of the number for the
             for name in c: # Loop through each OTU in a community
                 modularity_dict[name] = i # Create an entry in the dictionary for the OTU, where the value is which gro

         # Now you can add modularity information like we did the other metrics
         nx.set_node_attributes(G, modularity_dict, 'modularity')
```

```
In [30]: # add within-module degree attribute
         within_module_degree_dict = {}
         for i in G.nodes():
             neighbors = G.neighbors(i)
             module_members = [n for n in G.nodes() if G.nodes[n]['modularity'] == G.nodes[i]['modularity']]
             intersection = [v for v in neighbors if v in module_members]
             within_module_degree_dict[i] = len(intersection)

         # Now you can add modularity information like we did the other metrics
         nx.set_node_attributes(G, within_module_degree_dict, 'within_module_degree')
```

```
In [31]: # First get a list of just the nodes in that class
         class0 = [n for n in G.nodes() if G.nodes[n]['modularity'] == 0]
         # Then create a dictionary of the eigenvector centralities of those nodes
         class0_eigenvector = {n:G.nodes[n]['eigenvector'] for n in class0}
         # Then sort that dictionary and print the results
         class0_sorted_by_eigenvector = sorted(class0_eigenvector.items(), key=itemgetter(1), reverse=True)
         print("Modularity Class 0 Sorted by Eigenvector Centrality:")
         for node in class0_sorted_by_eigenvector:
             print("Name:", node[0], "| Eigenvector Centrality:", node[1])
```

```
Modularity Class 0 Sorted by Eigenvector Centrality:
Name: bradyrhizobium sp. | Eigenvector Centrality: 0.13206418339684925
Name: pleomorphomonas sp. | Eigenvector Centrality: 0.02577603033368898
Name: rhodopseudomonas spp. | Eigenvector Centrality: 0.024600617524410968
Name: magnetospirillum sp. | Eigenvector Centrality: 0.02409608769239579
Name: rhodopseudomonas palustris | Eigenvector Centrality: 0.022875920987163383
Name: rhodopseudomonas sp. | Eigenvector Centrality: 0.02025408463310669
Name: nitrobacter hamburgensis | Eigenvector Centrality: 0.01402070093274112
Name: afipia sp. | Eigenvector Centrality: 0.006015062116628816
Name: pleomorphomonas spp. | Eigenvector Centrality: 0.005722481456889898
Name: bradyrhizobium spp. | Eigenvector Centrality: 0.005217926734340098
Name: pleomorphomonas oryzae | Eigenvector Centrality: 0.0033440890172627877
Name: spirochaeta spp. | Eigenvector Centrality: 0.003220597233852982
Name: opitutus spp. | Eigenvector Centrality: 0.0028620944384821845
Name: rhodopseudomonas rhenobacensis | Eigenvector Centrality: 0.0028620944384821845
```

Indentification of Key Module Members

After all modules are separated, each node can be assigned a role based on its topological properties and the role of node i is characterized by its within-module connectivity (zi) and among-module connectivity (Pi)

Peripheral nodes: zi <= 2.5, Pi <= 0.62
Connector nodes: zi <= 2.5, Pi > 0.62
Module hub nodes: zi > 2.5, Pi <= 0.62
Network hub nodes: zi > 2.5, Pi > 0.62

Sources:

- https://www.researchgate.net/post/How_should_I_interpret_the_connectivity_measures_kTotal_kWithin_kOut_kDiff_in_WGCNA
- MENAP paper

```
In [32]: # add within-module connectivity attribute
         within_module_connectivity_dict = {}
         for i in G.nodes():
             b =  G.nodes[i]['modularity']
             k_ib = G.nodes[i]['within_module_degree']
             bs = [G.nodes[n]['within_module_degree'] for n in G.nodes if G.nodes[n]['modularity'] == b]
             k_bavg = np.mean(bs)
             k_bstd = np.std(bs)
             if (k_bstd == 0):
                 within_module_connectivity_dict[i] = 0
             else:
                 within_module_connectivity_dict[i] = (k_ib - k_bavg)/(k_bstd)

         nx.set_node_attributes(G, within_module_connectivity_dict, 'within_module_connectivity')
```

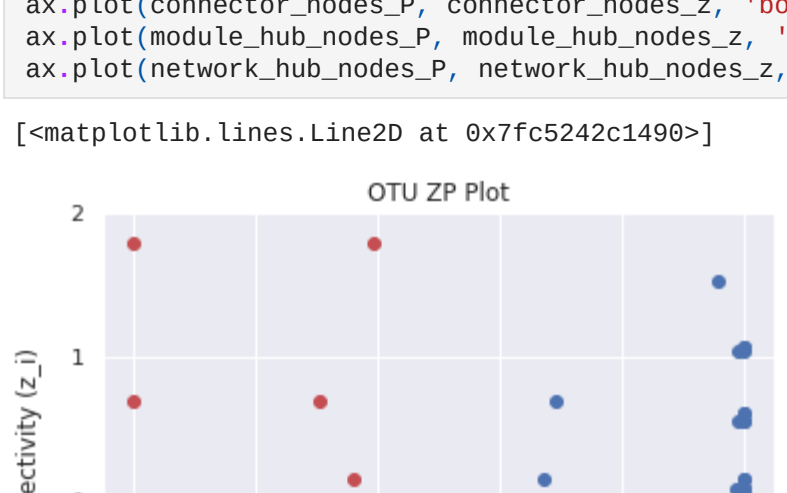
```
In [33]: # add among-module connectivity attribute
         among_module_connectivity_dict = {}
         for i in G.nodes():
             neighbors = G.neighbors(i)
             k_i = G.nodes[i]['degree']
             sum_k = 0
             for c in range(M):
                 module_members = [n for n in G.nodes() if G.nodes[n]['modularity'] == c]
                 intersection = [v for v in neighbors if v in module_members]
                 k_ic = len(intersection)
                 sum_k += (k_ic / k_i) ** 2
             among_module_connectivity_dict[i] = 1 - sum_k

         nx.set_node_attributes(G, among_module_connectivity_dict, 'among_module_connectivity')
```

```
In [38]: zt = 2.5
         Pt = 0.62

         peripheral_nodes_OTU = []
         connector_nodes_OTU = []
         module_hub_nodes_OTU = []
         network_hub_nodes_OTU = []

         peripheral_nodes_z = []
         connector_nodes_z = []
         module_hub_nodes_z = []
         network_hub_nodes_z = []

         peripheral_nodes_P = []
         connector_nodes_P = []
         module_hub_nodes_P = []
         network_hub_nodes_P = []

         for i in G.nodes():
             zi = G.nodes[i]['within_module_connectivity']
             Pi = G.nodes[i]['among_module_connectivity']
             print(i, "|", zi, "|", Pi)

             if zi <= zt and Pi <= Pt:
                 peripheral_nodes_OTU.append(i)
                 peripheral_nodes_z.append(zi)
                 peripheral_nodes_P.append(Pi)
             elif zi <= zt and Pi > Pt:
                 connector_nodes_OTU.append(i)
                 connector_nodes_z.append(zi)
                 connector_nodes_P.append(Pi)
             elif zi > zt and Pi <= Pt:
                 module_hub_nodes_OTU.append(i)
                 module_hub_nodes_z.append(zi)
                 module_hub_nodes_P.append(Pi)
             else:
                 network_hub_nodes_OTU.append(i)
                 network_hub_nodes_z.append(zi)
                 network_hub_nodes_P.append(Pi)

         print("---")
         print("len peripheral nodes:", len(peripheral_nodes_OTU))
         print("len connector nodes:", len(connector_nodes_OTU))
         print("len module hub nodes:", len(module_hub_nodes_OTU))
         print("len network hub nodes:", len(network_hub_nodes_OTU))
         print("total number of nodes:", len(G.nodes))
```

```
opitutus spp. | -0.9370425713316365 | 0.0
paludibacter propionicigenes | 0 | 0.96
magnetospirillum sp. | 1.7959982617189696 | 0.3950617283950617
rhodopseudomonas palustris | 1.7959982617189696 | 0.0
acetobacter spp. | -0.3067859955389483 | 1.0
bacteroides spp. | 1.5339299776947406 | 0.96
pleomorphomonas oryzae | -0.9370425713316365 | 0.0
afipia sp. | 0.156173761888606 | 0.6734693877551021
rhodoblastus acidophilus | 0 | 1.0
spirochaeta spp. | -0.9370425713316365 | 0.84
citrobacter werkmanii | 0 | 1.0
pontibacter spp. | 0.6135719910778962 | 1.0
pontibacter sp. | 0.15339299776947393 | 1.0
ruminococcus spp. | 0 | 0.96
eubacterium sp. | 1.044185127573248 | 1.0
desulfovibrio mexicanus | 0 | 1.0
ancalomicrobium spp. | 0 | 0.9876543209876543
rhodocyclus tenuis | -0.7669649888473705 | 0.9375
escherichia spp. | 0 | 1.0
rhodoplanes elegans | 0 | 0.9876543209876543
propionicimonas paludicola | 0 | 1.0
rhodopseudomonas spp. | 0.7027819284987272 | 0.30555555555555547
pleomorphomonas spp. | -0.39043440472151525 | 0.4375
caulobacter spp. | 0.5622535302317488 | 0.9917355371900827
opitutus terrae | 0 | 1.0
barnesiella vischericola | 0.15339299776947393 | 1.0
pontibacter salisaro | -1.687322975464215 | 1.0
phaeospirillum sp. | 0.0803219328902496 | 1.0
rhodopseudomonas pangongensis | 1.044185127573248 | 0.9930555555555556
enterobacter spp. | 0 | 1.0
prolixibacter spp. | -2.3293360538172467 | 1.0
cloacibacterium normanense | 0 | 1.0
rhodopseudomonas rhenobacensis | -0.9370425713316365 | 0.0
clostridium sp. | 0 | 0.96
pleomorphomonas sp. | 0.156173761888606 | 0.3599999999999999
citrobacter spp. | 0 | 1.0
bradyrhizobium sp. | 0.7027819284987272 | 0.691358024691358
acidovorax caeni | 0 | 1.0
fibrobacter spp. | 1.0737509843863184 | 1.0
chlorobium spp. | -1.2271439821557928 | 1.0
bradyrhizobium yuanmingense | 0 | 1.0
bradyrhizobium spp. | -0.39043440472151525 | 0.0
rhodopseudomonas sp. | 0.7027819284987272 | 0.0
inquilinus spp. | 1.0737509843863184 | 1.0
dechloromonas agitata | 0 | 1.0
acidovorax spp. | 0 | 1.0
opitutus sp. | 0.0803219328902496 | 0.99
methylocella palustris | 0 | 0.9876543209876543
beijerinckia spp. | 0 | 1.0
rhodocyclus spp. | -1.2271439821557928 | 1.0
ochrobactrum anthropi | 0.5622535302317488 | 1.0
nitrospirillum azospirillum amazonense | 0.0803219328902496 | 1.0
acinetobacter junii | 0 | 1.0
propionibacterium acnes | 0 | 0.9375
brevundimonas sp. | 0.0803219328902496 | 1.0
rhizobium petrolearium | -1.8474044564757475 | 0.9183673469387755
escherichia vulneris | 0.5622535302317488 | 1.0
brevundimonas spp. | 0.0803219328902496 | 1.0
parabacteroides distasonis | 0.6135719910778962 | 1.0
nitrobacter hamburgensis | -1.4836507379417576 | 0.0
---
len peripheral nodes: 11
len connector nodes: 49
len module hub nodes: 0
len network hub nodes: 0
total number of nodes: 60
```

```
In [35]: f, ax = plt.subplots(figsize=(6, 6))
         ax.set_title("OTU ZP Plot")
         ax.set_xlabel("among-module connectivity (P_i)")
         ax.set_ylabel("within-module connectivity (z_i)")
         ax.plot(peripheral_nodes_P, peripheral_nodes_z, 'ro')
         ax.plot(connector_nodes_P, connector_nodes_z, 'bo')
         ax.plot(module_hub_nodes_P, module_hub_nodes_z, 'bo')
         ax.plot(network_hub_nodes_P, network_hub_nodes_z, 'bo')
```

```
Out[35]: [<matplotlib.lines.Line2D at 0x7fc5242c1490>]
```



TODO: Turn This Network Analysis into separate analysis for each subsection of sample